University of Alberta

Drug-Disease Interactions: Effect of Inflammation on the Pharmacokinetics and Pharmacodynamics of Cardiovascular Drugs

by

Morvarid Noriko Daneshtalab ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the

requirements for the degree of *Doctor of Philosophy*

in

Pharmaceutical Sciences

Faculty of Pharmacy and Pharmaceutical Sciences

Edmonton, Alberta

Fall 2005

Library and
Archives Canada

Published Heritage
Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et
Archives Canada

Direction du
Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

0-494-08628-9

Your file *Votre référence*
ISBN
Our file *Notre reterence*
ISBN

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

There is freedom within
There is freedom without
Try to catch the deluge in a paper cup

There's a battle ahead
Many battles are lost
But you'll never see the end of the road
While you're traveling with Me
Composed by Neil Finn
Crowded House "Don't Dream It's Over"

Strange Studies…

STUDY:
DEAD PATIENTS
USUALLY NOT SAVED

Actual newspaper headline

From the calendar "365 Stupidest Sayings"

Dedications

To my mother and father, Emiko and Mohsen Daneshtalab
I thank you for everything. I am eternally grateful to God for your
presence in my life, always. You are my guiding lights.
May God keep you safe.

Also
To my much better half Daryl Pereira,
and my truest friend and 2nd family Nancy Dhillon;
For your enduring love, strength, and unquestioned support,
I am always thankful

Abstract

Inflammatory conditions decrease cardiovascular (CV) response to calcium channel blockers and β-adrenergic due, likely, to down-regulation of the receptors. Thesis objectives were: 1) A clinical investigation to determine the effect of angiotensin II type 1 receptor (AT$_1$R) antagonists in rheumatoid arthritis (RA). 2) Establish a suitable animal model to investigate the effect of inflammation on the pharmacodynamic (PD) response to CV drugs.

Subjects were divided into 3 groups: 14 with active RA, 12 with controlled arthritis and 12 healthy subjects. Single oral dose of AT$_1$R antagonists' valsartan (160 mg), and losartan (100 mg) were given separately. Serial blood samples were taken for pharmacokinetic (PK) and inflammatory mediator level analysis. Thirteen CV parameters were measured. Valsartan, losartan and the losartan active metabolite EXP 3174 were measured using HPLC.

Patients with active disease had significantly higher inflammatory mediator levels than other groups. PK of valsartan was unaltered. No reduced response to valsartan was noticed. No difference in losartan PK was observed in any of the groups. However, EXP 3174 bioavailability was significantly decreased in arthritic groups. Reduced levels of EXP 3174 were not accompanied by the expected down-regulation in clinical response.

Our preliminary observation suggested a need to establish an appropriate animal model to study the PD of CV drugs. ECG studies were performed on Sprague Dawley (SDR) (n = 8), Wistar Kyoto (WKY) (n = 11), and the Spontaneously Hypertensive rat (SHR) (n = 12) strains. Rats were divided into IFN $_{\alpha 2a}$ treated and control groups and

sotalol (80 mg/kg) was administered. The PR, RR, and QT, and QTc intervals were determined.

The positive control SDR responded to sotalol with PR and QT interval prolongation, as seen in humans. Decrease in responsiveness to sotalol was observed in inflamed groups in the SDR strain, as expected. Sotalol failed to prolong the PR interval in WKY and SHR strains. No difference was observed between controls and inflamed groups in WKY and SHR with PR interval. A decrease in β_1AR expression was suspected in the WKY and SHR. However, receptor expression was not significantly different among strains. SHR and WKY do not appear to be suitable animal models for the study of CV receptor downregulation under inflammatory conditions.

Acknowledgements

Before thanking any one person, I give my thanks to God for His presence in my life.

I would like to thank, first and foremost, my supervisor, Dr. Fakhreddin Jamali. I would not be the person and scientist I am now, if it were not for his constant guidance and support. I truly appreciate the effort it must have taken for him to be patient, and motivate and encourage me through my struggles. Thank you for your friendship and support.

Also, I would like to acknowledge and thank Dr. Richard Lewenczuk for his help and guidance in the losartan/valsartan study.

The Clinical Investigations Unit at University of Alberta Hospital also receives my thanks for their help and support with patient recruitment and study progression, especially Tina, Steven and Cinnamon. They brightened my every visit.

As well, I would like to thank Drs. Dion Brocks, Ayman El-Kadi, and William Dryden for their support and guidance.

To my good friends and colleagues past and present, some of whom helped shape my life in and out of the lab for the last few years, I give many thanks for their constant support and friendship: Dr. Ken Kulmatycki, Dr. Michael Guirguis, Dr. Tahereh Khazaeinia, Saeed Sattari, Yaman Dakhel, Kassem Abouchehade, Dr. Ali Aghazadeh, Spencer Ling, Sam Harirforoosh, John D Clements, and Nigel Dagenais.

Finally, I would like to acknowledge John Priegert Shihan for his support, friendship, and faith during the toughest times. Thank you.

Table of Contents

Title **Page**

Chapter 1: Introduction 1

Inflammation 3
Pathogenesis of Rheumatoid Arthritis 4
Cardiovascular risk profile in RA patients 5
Soluble & Cellular Mediators of Inflammation: The Th1/Th2 balance 8
Cytokines involved in Th1/Th2 balance 11
Pro-inflammatory cytokines 14
    TNFα 15
    IL-1 17
    IL-6 18
    Interferon (IFNα) 19

Cytokine Stimulated Inflammatory Mediators

    C – Reactive Protein 21
    Inducible Nitric Oxide Synthase 24
Inflammatory actions of Angiotensin II (AGII) 25
Molecular Biology of AT$_1$R and AT$_2$R 26
ACE and AT$_1$R Inhibition: Therapeutic Relevance 30
Losartan Pharmacokinetics and Pharmacodynamics 32
Valsartan Pharmacokinetics and Pharmacodynamics 35
Clinical Applications of Losartan and Valsartan 37

Influences of Pro-inflammatory Mediators on Drug Pharmacokinetics

    Absorption 39
    Hepatic Excretion and Transport Proteins 41
    Metabolism 42
    Plasma Protein Levels 45

Influences of Pro-Inflammatory Mediators on Drug Pharmacodynamics

    G ProteinCoupled Receptor (GPCR) Activation 46
    GPCR Regulation: Implications in CVD and Inflammation 47
    Cross-talk between Receptors and Receptor Oligomerization 49

Chapter 1: Introduction Continued

Objectives of the Clinical Investigation 52
Hypotheses for the Clinical Investigation 53
Hypertensive Rat Model Strains 54
Objectives for the Animal Study 57
Sotalol pharmacokinetics and pharmacodynamics 57
Clinical application of Sotalol 58

Hypotheses for the Animal Study 60

Chapter 2: Experimental

Assays for Pharmcokinetic Determination 61
  HPLC Apparatus 61
  Assay for Valsartan 61
    Sample preparation 62
    Quantification 62
    Extraction efficacy, accuracy, and precision 63
  Assay for Losartan 66
Inflammatory Determination Assays
    Nitric Oxide determination 67
    C-Reactive Protein determination 67
    Segmented Neutrophil count 68
Human Clinical Study Protocol
  Ethics and Consent 69
    Inclusion Criteria 69
    Exclusion Criteria 70
  Subject Selection and Protocol Summary 71
  Power of the Study 74
  Statistical Analysis for the Human Study 74
Rat Study Protocol
  Animals and dosing 75
  Induction of Inflammation 75
  Drug Administration 76
  Electrographic Measurements and Analysis 77
  Western blot techniques
    Homogenization of the rat hearts 80
    β-AR Protein Density Level Determination 81
  Statistical Analysis for the Rat Study 82

Chapter 3: Human Study Results and Discussion

Results:
  Subject Characteristics 84
  Inflammatory Mediator levels 84
  Valsartan Pharmacokinetics 90
  Losartan Pharmacokinetics 92
  Pharmacodynamic Characteristics
    Valsartan Pharmacodynamics 97
    Losartan Pharmacodynamics 103
Discussion: 110

Chapter 4: Strain Difference in Response to Sotalol. Results and Discussion

Results:

Inflammatory State 124

Pharmacodynamics

Effect of Interferon on ECG Parameters 127

Effect of Sotalol on ECG Parameters 127

Effect of Sotalol between Control and Inflamed Groups 128

Strain Difference in ECG parameters 128

Western Blot Analysis 129

Discussion: 141

Chapter 4: General Conclusion 149

References: 152

List of Tables

Title **Page**

Chapter 1

Table 1-1:
Similarities in inflammatory response between atherosclerosis and RA 7
Table 1-2:
Subtypes of $CD4^+$ T helper cells and their characteristic cytokines and effects 9
Table 1-3:
Functional consequences of GPCR heterodimerization 50

Chapter 2

Table 2-1:
Accuracy (% error) and precision (coefficient of variation, % CV) of valsartan spiked solutions 64

Chapter 3

Table 3-1:
Characteristics of subjects. Mean± SD presented 86
Table 3-2:
Mean± SD of Inflammatory mediator characteristics of subjects 88
Table 3-3:
Mean ± SD of valsartan PK indices 91
Table 3-4:
Mean ± SD of losartan and EXP 3174 PK indices 94
Table 3-5:
Mean± SEM of valsartan Pharmacodynamics as expressed in area under the percent change in effect from baseline times time (AUEC) 98
Table 3-6:
Mean± SD of maximum effect with valsartan treatment as expressed as percent change from baseline 99
Table 3-7:
Mean± SEM of losartan Pharmacodynamics as expressed in area under the percent change in effect from baseline times time (AUEC) 104
Table 3-8:
Mean± SD of maximum effect with losartan treatment as expressed as percent change from baseline 105

Chapter 4

Table 4-1:
Mean± SD of percent segmented neutrophil levels after 1st and 2nd injections of saline or $IFN_{\alpha 2a}$ 141
Table 4-2:
Mean± SD of $AUEC_{0-3h}$ for RR, QT, PR, and QTc intervals after saline or $IFN_{\alpha 2a}$ injections 130
Table 4-3:
Mean± SD of maximum %change in baseline ECG parameters before and after sotalol treatment among strains 131
Table 4-4:
Mean ± SD of $AUEC_{0-4h}$ for RR, QT, PR and QTc intervals after sotalol treatment 137
Table 4-5:
Mean± SEM of baseline difference in ECG parameters among strains 138

List of Figures

Title **Page**

Chapter 1

Figure 1-1:
The balance of Th1//Th2 cytokines in rheumatoid arthritis 9
Figure 1-2:
Cytokine cascade of increasing TNFα levels in rheumatoid arthritis 16
Figure 1-3:
Pathway of CRP production and effect as a pro-inflammatory mediator 23
Figure 1-4:
AGII mediated effects via stimulation of AT_1R and AT_2R 27
Figure 1-5:
CYP 450 oxidation of losartan 33
Figure 1-6:
Structure and metabolic pathway of valsartan 36

Chapter 2

Figure 2-1:
Example of valsartan Chromatograph 65
Figure 2-2:
Electrode placement for the rat ECG 78
Figure 2-3:
Typical rat ECG 78
Figure 2-4:
Experimental protocol summary for the rat experiment 79

Chapter 3

Figure 3-1:
Mean± SD of markers of inflammation: AI, CRP and NO 87
Figure 3-2:
Correlation graphs between AI and CRP, AI and NO_2^-, and CPR and NO_2^-. 89
Figure 3-3:
Mean± SD of plasma valsartan concentration versus time curves 91
Figure 3-4:
Mean± SD of plasma losartan and EXP 3174 concentration versus time curves 93
Figure 3-5:
Mean± SD of AUC for losartan and EXP 3174 for the three groups of subjects 95
Figure 3-6:
Abnormal subject data 96
Figure 3-7:
Concentration versus effect from baseline for SBP, DBP, MAP with valsartan treatment 100
Figure 3-8:
Effect of a single 160 mg oral dose of valsartan on blood pressure 101
Figure 3-9:
Percent change in effect from baseline versus time for SBP, DBP, and MAP for valsartan 102
Figure 3-10:
Concentration versus effect from baseline for SBP, DBP, MAP with losartan treatment 106
Figure 3-11:
Concentration versus effect from baseline for SBP, DBP, MAP with EXP 3174 treatment 107
Figure 3-12:
Effect of a single 100 mg oral dose of losartan on blood pressure 108

Figure 3-13:
Percent change in effect from baseline versus time for SBP, DBP, and MAP for losartan 109

Chapter 4

Figure 4-1:
Mean± SD of baseline percent segmented neutrophil counts among the three strains 126
Figure 4-2:
Mean± SD of time course of sotalol percent effect from baseline following administration of single oral dose of 80 mg/kg sotalol for SDR 132
Figure 4-3:
Mean± SD of time course of sotalol percent effect from baseline following administration of single oral dose of 80 mg/kg sotalol for WKY 133
Figure 4-4:
Mean± SD of time course of sotalol percent effect from baseline following administration of single oral dose of 80 mg/kg sotalol for SHR 134
Figure 4-5:
Mean± SD of response to sotalol in control versus IFN$_{\alpha 2a}$ treatment among rat strains. AUEC $_{0\text{-}6h}$ was depicted for heart rate (RR) and PR 135
Figure 4-6:
Mean± SD of response to sotalol in control versu[illegible]N$_{\alpha 2a}$ treatment among rat strains. AUEC $_{0\text{-}6h}$ was depicted for QT and QTc 136
Figure 4-7:
Mean± SD of response to sotalol in inflamed and non-inflamed groups between strains. Heart rate (RR), QT and PR intervals are presented as AUEC $_{0\text{-}6h}$ after sotalol treatment 139
Figure 4-8:
Western blot analysis of β_1AR protein expression in whole heart 140

List of Abbreviations and Symbols

AC	Adenylate cyclase
ACE/ACEI	Angiotensin converting enzyme/ACE inhibitor
AGI/AGII	Angiotensin I/II
AI	Arthritic index
ANOVA	Analysis of variance
AP-1	Apo-protein 1
AT_1R/AT_2R	Angiotensin II type 1/type2 receptor
AUEC	Area under the % effect from baseline versus time curve
AUC_{0-t}	Area under the concentration versus time curve
β_1AR	Beta-1 adrenergic receptor
βARK	Beta adrenergic receptor kinase
Bcl-2	B-cell lymphoma-2
Bmax	Maximum binding
CD4$^+$	Cluster of differentiation cells 4
β	Terminal half-life
Cmax	Maximum concentration
Card ej t	Cardiac ejection time
cDNA	Chromosomal deoxyribonucleic acid
Clcr	Creatinine clearance
Cl/F	oral clearance
CHF	Cardiac heart failure
Ci	Cardiac output index
cAMP	Cyclic adenosine mono-phosphate
cGMP	Cyclic guanidine monophosphate
Co	Cardiac output
COX-2	Cyclooxygenase 2
CRP	C-reactive protein
CV	Cardiovascular
CVD	Cardiovascular Disease
CYP 450	Cytochrome P450
DBP	Diastolic blood pressure
DTH	Delayed type hypersensitivity
ECG	Electrocardiograph
ELISA	Enzyme-linked immuno-sorbent assay
Emax	Maximum effect
ERK	Extra-cellular signal-Regulated Kinase
FAD	Flavin adenine di-nucleotide
Fc	Functional component
GDP	Guanosine di-phosphate
GIT	Gastro-intestinal tract
GPCR	G-protein coupled receptor
GRK	G-protein coupled kinase
Gs, Gi, Gq	heterotrimeric stimulatory/inhibitory/q guanine nucleotide binding protein
GTP	Guanosine tri-phosphate
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
HPLC	High performance liquid chromatograph
HRP	Horse-radish peroxidase
ICAM-1	Intercellular adhesion molecule-1
IL (various)	Interleukin
IFN	Interferon
IgG, E	Immunoglobulin G,F

iNOS	inducible nitric oxide synthase
iv	intravenous
JAK/STAT	Janus activated kinase/signal transducer and activator of transcription
KDa	Kilo Daltons
LDH	Lactate dehydrogenase
LE	Large artery compliance
LPS	lipopolysaccharide
LT	Lymphotoxin
LVH	Left ventricular hypertrophy
MAP	Mean arterial pressure
MAPK	Mitogen Activated Protein Kinase
MCP-1	Monocyte chemoattractant protein-1
MDR	Multi-drug resistant
MHC Class II	Major histocompatibility complex Class II
MI	Myocardial Infarction
cMOAT	Canalicular multispecific organic anion transporter
mRNA	Messenger ribonucleic acid
NADPH	Nicotinamide adenine di-phosphate
NFκ B	Nuclear Factor κ B
NK cells	Natural killer cells
NO	Nitric Oxide
NO_2	Nitrite
NO_3	Nitrate
OATP	Organic anion transporting polypeptide
$O_2^{\cdot}$	superoxide
$ONOO^-$	Peroxinitrite
OP2	Opioid Receptor 2
PAF	Plasminogen activating factor
PBMC	Peripheral blood mononuclear cells
Pgp	P-glycoprotein
PKA	Protein kinase A
PKC	Phosphokinase C
PK/PD	Pharmacokinetic/pharmacodynamic
PGE_2,$PGF_{2\alpha}$	Prostoglandin E_2, $F_{2\alpha}$
PLC β,γ1	Phospholipase C β,γ1
po	Per oral
PSM	Pre-stained marker
PVR	Peripheral vascular resistance
RA	Rheumatoid arthritis
RAS	Renin-angiotensin system
RES	Reticulo-endothelial system
ROS	Reactive oxygen species
Rpm	Rotations per min
SBP	Systolic blood pressure
SDR	Sprague-Dawley Rat
SE	Small artery compliance
SHR	Spontaneously hypertensive Rat
SD	Standard deviation
SDS-PAGE	Sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SLE	Systemic lupus erythematosus
SR	Sarcoplasmic reticulum
Sv	Stroke volume
Svi	Stroke volume index
$t_{1/2}$	Half-life
TMB	3,3'5,5-tetramethylbenzidine base
Th1,2,0	T-helper cell 1,2, 0

Tmax	Maximum time
TNFα, β	Tumor necrosis factor α, β
TPBS	Tween 20-Phosphate buffered saline
TVI	Total vascular impedance
TXA_2	Thromboxane A2
UV	Ultra-violet
VSM	Vascular smooth muscle
Vd	Volume of distribution
Vd/F	oral volume of distribution
WKY	Wistar Kyoto Rat

Chapter 1: Introduction

Inflammatory conditions are associated with increases in various pro-inflammatory mediators (e.g., interleukins, tumor necrosis factor α, C-reactive protein and nitric oxide) often proportionate to the severity of disease (Maury., 1989; Lepore et al., 1994; Ueki et al., 1996). Many cardiovascular conditions such as hypertension, congestive heart failure and myocardial infarction (Saito et al., 2003; Peeters et al., 2001; Lieu et al., 1999; Westhuyzen et al., 2000) are also associated with increased pro-inflammatory mediators. Indeed, elevated pro-inflammatory cytokines and C-reactive protein (CRP) have been suggested to be independent risk factors for atherosclerotic vascular disease and myocardial infarction (Balbay et al., 2001; Carlstedt et al., 1997; Sung et al., 2003; Rosenson et al., 2002; Griselli et al., 1999). In keeping with this hypothesis is the increased mortality due to development of cardiovascular complications seen in patients with rheumatoid arthritis, a disease in which there is increase in pro-inflammatory mediator levels (Prior et al., 1984; Mutru et al., 1985; Myllykangas-Luosujarvi et al., 1995; Wolfe et al., 1994).

Pro-inflammatory mediators have also been shown to reduce clearance of efficiently metabolized drugs through physiological alterations, leading to altered drug absorption, distribution, metabolism and elimination (Morgan., 1997). A resulting increased drug plasma concentration is observed. Interestingly, this elevation of drug concentration is associated with decreased rather than increased potency of, at least, selected cardiovascular drugs such as calcium channel blockers in patients with rheumatoid arthritis (Mayo et al., 2000) as well as β-adrenergic antagonists and potassium channel blockers in animal models of arthritis (Kulmatycki et al., 2001; Guirguis et al., 2003). These effects are attributed to possible decrease in receptor expression, signaling efficiency, or altered downstream signaling mechanisms due to increases in pro-inflammatory mediators. A reduced effectiveness of cardiovascular drugs, therefore, may, at least in part, be contributing to the higher rate of mortality in states with concomitant cardiac disease and inflammation, and it is important to understand whether the down-regulating effect is universal to all cardiovascular drugs during hyper-inflammatory states.

The purpose of the human study was to investigate whether the potency of angiotensin II type 1 receptor (AT_1R) antagonists valsartan and losartan, with varying pharmacokinetic profiles, are also reduced by inflammation. The clearance of valsartan does not depend upon hepatic metabolism; hence it is unlikely to be affected by inflammation. The possibility of altered pharmacodynamics can, therefore, be investigated in the absence of complications arising from altered pharmacokinetics. As well, losartan pharmacokinetics and pharmacodynamics in the presence of inflammation was examined. Similar to valsartan, losartan is an AT_1R antagonist. However, while valsartan is a pharmacologically active compound (Müller et al., 1997), losartan, on the other hand, is converted to the more active metabolite EXP 3174 via cytochrome P450 (CYP) enzymes (Lo et al., 1994). The extent of this conversion may be reduced by inflammation leading to reduced effect. To elucidate further the mechanism of down-regulation observed with varying cardiovascular drugs, an appropriate animal model, reflecting the true disease state of the human patient taking antihypertensive drugs, is also necessary. So far, the rat models used did not incorporate the hypertensive state in an inflammatory model. Cardiovascular (CV) complications such as hypertension exhibit an imbalance between the pro- and anti-inflammatory cytokine profile, and altered electrophysiology of the heart. The neuro-endocrine release profile is also altered, leading to the exasperation of the inflammatory state. To understand the pharmacodynamic profile of cardiovascular drugs in cardio-inflammatory states, we also decided to see whether we can provide a more suitable inflammatory disease model with the spontaneously hypertensive rat (SHR) and its normotensive counterpart, Wistar Kyoto rat (WKY). The objective of the rat study was to compare the effect of inflammation on the pharmacodynamics of sotalol on the electrocardiograph (ECG) parameters of the above-mentioned strains to the existing, established inflammatory Sprague Dawley rat model and determine the suitability of the SHR strain as the human model for cardio-inflammatory disease study.

We chose rheumatoid arthritis as our human model of inflammation since it is associated with an increase in cardiovascular events and because our previous studies on down-regulation of calcium channel receptors revealed abnormalities in this group of subjects. The SHR and WKY rat strains were chosen due to their extensive use in the medical field as good models to study CV abnormalities. Before discussing the rationale and objectives of this thesis, background will be provided.

Inflammation

The host immune system consists of individual immune cells, immune cell aggregates, immune tissues and immune organs designed to act as the body's defense mechanism. The inflammatory process, in its basic form, has the capacity to respond specifically to foreign antigens and develop an enhanced response to encountered antigens during the first exposure (priming). As well, it should be able to retain specific memory of an encountered antigen and respond quickly to second exposure and to distinguish between self and non-self (Textbook of Rheumatology 1997).

The system is able to perform the above-mentioned duties using a great variety of diffusible substances to convey messages, give instruction, and generally enable the billions of immune cells to communicate with each other. However, autoimmune diseases arise when immune cells attack the body's own tissues without distinction.

Pathogenesis of Rheumatoid Arthritis

Rheumatoid arthritis (RA) is the most common autoimmune chronic inflammatory and destructive arthropathy, affecting about 1 % of the general population worldwide (Choy et al., 2001; Textbook of Rheumatology 1997). Incidence of RA is constant across the globe, with the exception of the Chinese (< 0.3 %) or the Pima Indians in North America (~ 5 %) (Textbook of Rheumatology., 1997). It is a disease of the joints, and is characterized by polyarthritis and a variety of extra-articular manifestations in more severe cases (Turesson et al., 2004; Feldman et al., 2001). This is indicative of a systemic disease, with the long-term prognosis being poor: 80 % of affected patients are disabled after 20 years (Scott et al., 1987), and life expectancy is reduced by an average of 3-18 years (Pincus et al., 1986; Sattar et al., 2003). The synovial membrane in patients with RA is characterized by hyperplasia, increased vascularity, and an infiltrate of inflammatory cells, primarily CD4$^+$ T cells, which are the main orchestrators of the cell-mediated immune response (Choy et al., 2001). Damage to the extracellular matrix is a feature of RA, with degradation of ligaments, tendons, and bone. In genetic studies, RA is strongly linked to the MHC Class II antigens HLA-

DRB1*0404 and DRB1*0401 (Lanchbury JS., 1992; Dedhia et al., 2002). The main function of HLA class II molecules is to present antigenic peptides to CD4$^+$ T cells, and unidentified arthritogenic antigens are strongly suspected.

Cardiovascular Risk profile in RA patients

Considerable evidence indicates that patients with RA are at greater risk of developing CV disease. In fact, the most common cause of death in patients with RA is due to cardiovascular diseases, accounting for 35-59 % of excess mortality in RA patients (Mutru et al., 1989; Myllyknagas-Luosujarvi et al., 1995). Coronary heart diseases appear to be prevalent, with pericardial effusion being the most frequent cardiac complication (Carrao et al., 1995). Myocardial inflammation, coronary vasculitis, as well as myocardial and endocardial functional abnormalities are also found in the autopsies of RA patients (Morris., 1986, Carrao et al., 1995, 1996; Cindas et al., 2002). Further epidemiological evidence can be observed in a prospective cohort study conducted among 114, 343 women in the Nurses' Health study who were free of CV diseases (CVD) and RA in 1976. It was found that women with RA had more than twice the risk of myocardial infarction compared with women without RA (Solomon et al., 2003). Most evidence suggests that classic risk factors do not explain excess CV disease in RA in the investigations. In an 8-year follow-up of 236 RA patients, a 3.96-fold higher incidence of CV events relative to a community-dwelling cohort was noted (del Rincón et al., 2001). In fact, disease severity has been associated with CV mortality in RA. It is becoming more evident that systemic markers of inflammation independently predict cardiac heart disease (CHD) events in men and women with or without existing heart disease (Libby et al., 2002; Pearson et al., 2003).

Cardiovascular diseases, such as atherosclerosis, were considered to be a process of aging, hyperlipidemia, or the fibroproliferative response of the vessel wall to the initial lipid damage. More recently, the inflammation hypothesis appears to be taking hold, as the production of adhesion molecules and chemokines due to an initial assault result in increasing inflammatory response, very similar to the systemic inflammatory response observed in RA (Table 1-1). Indeed, RA is not the only chronic inflammatory disease that is associated with CV disease. Systemic lupus erythematosus is also characterized by a high rate of coronary artery disease, mediated in part by chronic inflammation (Manzi et al., 1997). Factors that underlie this increased risk of myocardial infarction (MI) and CVD in RA patients appear to be increased inflammatory mediator presence and activity, as seen in atherosclerosis. Recent studies provide evidence that pro-inflammatory cytokines and inflammation markers: CRP, fibrinogen and erythrocytes sedimentation rate (ESR) are associated with cardiovascular disorders in humans (Verheggen et al,, 1999; Lagrand et al., 1999). Many of the cells comprising the inflammatory infiltrate in the joint lining are likewise found in atherosclerotic plaques. Evidence points more and more to the imbalance in the T-helper 1 (Th1) or T-helper 2 (Th2) cytokines and the increasing pro-inflammatory mediators, shared between RA and cardiovascular disorders. Mediators such as CRP and nitric oxide (NO), are thought to be the instigators promoting accelerated atherogenesis and CV diseases in RA (Popa et al., 2004; Weyand et al., 2001; Ridker et al., 1998). Additionally, therapies such as methotrexate and prednisone, both potent immunosuppressives, as well as anti- tumor necrosis factor (TNF) α therapies

Table 1-1: Similarities in inflammatory response between atherosclerosis and rheumatoid arthritis. Adapted from Yeh ETH: CRP as a mediator of disease. *Circulation* 2004; 109 [suppl II]: II-1 1-II-14

Similarities Between Atherosclerosis and Rheumatoid Arthritis

	Atherosclerosis	**Rheumatoid Arthritis**
Macrophage Activation	↑	↑
TNFα	↑	↑
Metalloproteinase expression	↑	↑
IL-6	↑	↑
Mast cell activation	↑(UA)	↑
T-cell activation	↑(UA)	↑
Soluble IL-2 receptor	↑(UA)	↑
CD3$^+$ DR$^+$	↑(UA)	↑
CD4$^+$ CD 28	↑(UA)	↑
CD4$^+$ IFN-γ$^+$	↑(UA)	↑
Th1/Th2 balance	↑Th1	↑Th1
B-cell activation		
Autoantibodies (ox-LDL, HSP)	0 or ↑	0 or ↑
Rheumatoid factor	0	↑
C-reactive protein	↑ (UA)	↑↑
Adhesion molecules (VCAM-1, ICAM-1, E-selectin, P-selectin)	↑	↑
Endothelin	↑	↑
Neoangiogenesis	↑	↑
Possible antigens	HSP, ox-LDL, infectious agents	Collagen II, cartilage antigens HSP, infectious agents

↑indicates increase; ↑↑,marked increase: HSP, heat shock protein; ICAM-1, intercellular adhesion molecule; IFNγ, interferon-gamma; IL-2, interleukin-2; IL-6, interleukin-6; ox-LDL, oxidized low-density lipoprotein; TNF-α, tumor necrosis factor-alpha; US, systemic markers found increased in patients with unstable angina; and VCAM, vascular adhesion molecuire. Other factors are expressed in atherosclerotic plaques.

have been found to be associated with a reduced rate of cardiovascular mortality. Improved endothelial function with decreases in pro-inflammatory mediators is observed in patients with RA with anti-inflammatory treatments (Choi et al., 2002; Hürlimann et al., 2002; Popa et al., 2004).

Soluble & Cellular Mediators of Inflammation: The Th1/Th2 balance

Cytokines are soluble protein or glycoprotein molecules secreted by a variety of cells in response to a challenge. Their primary roles are to regulate the growth, differentiation and function of cells in an autocrine, paracrine and endocrine manner. Specific cytokines have been dubbed being pro-inflammatory, anti-inflammatory, and growth factors. Interestingly, the challenge of the immune system is being able to co-ordinate the pro- and anti-inflammatory cytokines in a homeostatic manner. They are involved primarily in regulating immune and inflammatory response. The cytokine secretion patterns and effector functions rest largely in the T-helper cells. In particular the $CD4^+$ T cells can be divided into at least three different subsets (Table 1-2, Figure 1). All T lymphocytes start as naïve Th0 cells, which, after being activated, are able to polarize into either Th1 or Th2 cells. Th1 develop preferentially during infections with intracellular bacteria and raise the classic delayed-type hypersensitivity (DTH) skin response to viral and bacterial antigens, and fight cancer cells. Upon activation, Th1 secrete the pro-inflammatory cytokines interleukin (IL-2), interferon (IFN) γ and lymphotoxin (LT) α and TNFβ. They then activate macrophages to produce reactive oxygen intermediates and NO, stimulate their phagocytic functions and enhance their ability for antigen presentation by up-regulation of major histocompatibility complex (MHC) class II molecules (Schulze-Koops et al., 2001). Moreover, the Th1 cells promote

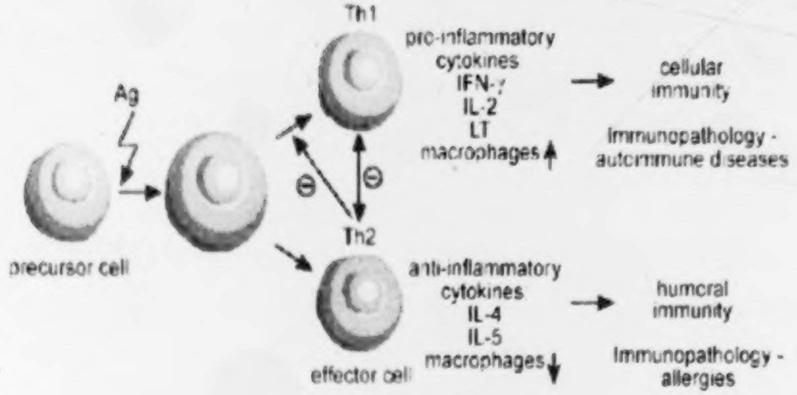


Figure 1-1: The balance of Th1/Th2 cytokines in rheumatoid arthritis. Adapted from Schulze-Koops H, Kalden JR. The balance of Th1/Th2 cytokines in rheumatoid arthritis. Best Practice and Research *Clin Rheumatol* 2001; 15: 677-691.

Table 1-2: Subtypes of CD4$^+$ T-helper cells and their characteristic cytokines and effects

Subtype	Defining cytokines	Effects(s)
Naïve Th0	*IL-2*	*Proliferate and differentiate to effector cells*
Th1	*IL-2, IFN γ, Lymphotoxin-α*	*Cell-mediated immunity, opsonizing antibody, Pro-inflammatory*
Mature Th0	*IFN γ, IL-4, others*	*Unclear*
Th2	*IL-4, IL-5, IL-9, IL-10, IL-13*	*Humoral immunity, Anti-inflammatory*
T-regulatory 1	*High levels of IL-10, some TGF-β*	*Suppress immune response; anti-inflammatory*

T-regulatory 1 cell may represent one form of the long-elusive T-suppressor cell. Recent data have implicated both CD4$^+$ T-regulatory 1 cells and a separate population of CD4$^+$ CD25$^+$ T-helper cells in antigen-specific immunosuppression.

Adapted from Spellberg B, Edwards JR: Type1/Type 2 Immunity in Infectious Diseases. *Clin Inf Dis* 2001; 32: 76-102.

the induction of complement-fixing, opsonizing antibodies and of antibodies involved in antibody-dependent cell cytotoxicity, such as IgG1 in humans. They also stimulate adhesion molecule expression on endothelial cells and induce endothelial cell retraction and vascular smooth- muscle relaxation. The result is the accumulation of blood in dilated, leaky vessels, easing diapedesis of leukocytes into areas of danger and allowing recruitment of innate immune cells, leading to the 4 cardinal signs of inflammation. Both IFNγ and LT α induce other cell types, including non-leukocytes such as endothelial cells (Goebeler et al., 1997) and fibroblasts (Rathanaswami et al., 1993; Teran et al., 1999) to secrete pro-inflammatory cytokines such as TNF and chemokines (Nickoloff et al., 1994). Consequently, the Th1 cells have been implicated in cell mediated immunity, or type 1 immunity (Spellberg et al., 2001).

Th2 cells conversely stimulate high titers of antibody production. They predominate after parasitic infections such as gastrointestinal nematodes or helminthes. Cytokines secreted by Th2 include the anti-inflammatory cytokines IL-4, IL-5, IL-9, IL-10, and IL-13. They provide potent help for B cell activation and immunoglobulin class switching to IgE and subtypes of IgG. They are known to mediate allergic and atopic reactions, as well as causing airway inflammation seen in asthma and reactive airway disease (Robinson et al., 1993; Shi et al., 1998; Li et al., 1999). Type 2-mediated inflammation is characterized by eosinophilic and basophilic tissue infiltrations, as well as extensive mast cell degranulation, dependent on cross-linking of surface-bound IgE. The anti-inflammatory actions of IL-4 also inhibit macrophage activation. It appears some cytokines, such as IL-6, are produced by both types of T-helper cells (Spellberg et al., 2001).

There are five factors inducing polarization of T-helper cells to Th1 or Th2 type immunity, depending on the necessity of the immune protection. The main factor is the local cytokine milieu. Other four factors are the presence of immunologically active hormones, the dose and route of antigen administration, the type of antigen presenting cell stimulating the T cell, and the "strength of signal" or the affinity of T-cell receptor for the MHC-antigen complex.

Cytokines involved in Th1/Th2 Balance

IL-12 is the key cytokine allowing polarization into the Th1 phenotype (Seder et al., 1993; Hsieh et al., 1993), while IL-4 mediates Th2 polarization (Swain et al., 1988; Betz et al., 1990). Th1 or Th2 polarization may not occur until an activated T cell arrives at the site of danger and samples the local cytokine milieu to determine the appropriate immune response. Hence the phenotype of newly activated T cell is determined within 48-72 h after activation (Sornasse et al., 1996). Importantly, Th1 and Th2 cells cross-regulate each other by blocking the generation of the antipodic cell type and by blocking each other's effector functions. Increasing IFN γ secreted by Th1 cells will directly suppress IL-4 secretion and thus inhibit differentiation of naïve Th0 cells to Th2 cells. (Gajewski et al., 1988 (140, 141)). Conversely, IL-4 and IL-10 inhibit the secretion of IL-12 and IFNγ, thus blocking the ability of Th0 cells to polarize into Th1 cells (D'Andrea et al., 1993; Ohmori et al., 1997). Differentiation of the appropriate T-helper subset is crucial to the host in mounting protective immunity against an exogenous microorganism. However, immune responses driven preferentially by activated Th subsets are also involved in the development of pathological auto-immune disorders. Whereas atopic disease result from Th2-dominant response to allergens, Th1-mediated immunity is involved in the generation of several organ-specific experimental autoimmune diseases, such as experimental allergic encephalomyelitis, insulin-dependent diabetes mellitus and collagen-induced arthritis (Spellberg et al., 2001). Molecular genetic modification of the Th0 naïve cells may also lead to preferential polarization to either Th1 or Th2 cells. Ligation of the IL-12 receptor on the Th0 cells activates the transcription factor STAT 4, which triggers regulatory sequences leading to Th1 polarization (Bacon et al., 1995). Activating STAT 6 by the ligation of the IL-4 receptor on the Th0 cells will, in turn, lead to Th2 polarization (Hou et al., 1994).

Indeed, experimental and epidemiological evidence suggest a dominant Th1 drive in RA. For several decades, the ameliorating effects of pregnancy on the course of RA have been noted (Da Silva et al., 1992). Interestingly, a marked decrease in Th1-mediated immunity is evident, as pregnant women have a higher incidence of infections, in particular, infections with intracellular pathogens. Pro-inflammatory IFNγ levels are also reduced and the anti-inflammatory IL-4 and IL-5 levels increase (Shulze-Koops et al., 2001). There is also a decreased prevalence of allergic diseases in patients with RA, with the prevalence of hay fever being significantly lower in RA than appropriate controls (4 versus 8 %) (Verhoef et al., 1998). Exogenous cytokines have been used increasingly for treatment of several different malignancies and viral infections. They also provide an excellent opportunity to explore the effect of cytokines on T cell function and differentiation after *in vivo* application with regard to autoimmunity. In an attempt to enhance anti-tumor cellular cytotoxicity, IL-12 was administered to a woman with metastatic cervical cancer, and a severe exacerbation of her RA was noted. IL-12 is a strong inducer of Th1 cell development with subsequent IFNγ production. In RA, the prevalence of Th1 drive is evident within the T cells cloned from the human rheumatoid synovial membrane (Schulze-Koops et al., 2001). Out of a panel of 19 synovial membrane derived T cell clones, 18 produced large amounts of IFNγ whereas IL-4 was absent. The markedly elevated Th1/Th2 ratio in the synovial fluid also correlated with disease activity (Van der Graaff et al., 1999). Similarly, a drastically reduced IL-4 and IL-10 mRNA synthesis in mononuclear cells of RA patients correlated with disease activity (Miyata et al., 2000).

Altogether, it seems evident that in RA, the autoimmunity is driven by activated Th1 effectors without sufficient Th2 generation to down-regulate inflammation. Selective manipulation of Th cell differentiation to induce Th2 effector anti-inflammatory cytokines might be a successful approach in interrupting ongoing and established Th1-driven chronic autoimmune diseases.

In RA, the primary site of inflammation is synovial tissue, from which cytokines can be released into systemic circulation. Measurable plasma levels of TNFα, IL-1β, and IL-6 and matrix metalloproteinases are commonly present at several-fold higher levels than noted, as antigen-activated CD4$^+$ T cells stimulate monocytes, macrophages, and synovial fibroblasts to release these inflammatory cytokines. Increasing CRP levels have also been implicated in RA patients, and constitute one of the acute phase proteins inducing the complement system (Plant et al., 2000; Hirschfield et al., 2003). It is therefore important to take a look at the various pro-inflammatory cytokines and inflammatory mediators important in disease determination and progression, as well as investigate their involvement in the pathophysiological changes leading to the altered PK/PD of drugs.

Pro-Inflammatory Cytokines

Some cytokines function primarily to induce inflammation, while others suppress inflammation. This concept is based on genes coding for the synthesis of mediator molecules up-regulated in inflammation, such as type II phospholipase (PL) A2, cyclooxygenase 2 (COX 2), and inducible NO synthase (Dinarello et al., 2000). These genes code for enzymes that increase the synthesis of platelet-activating factor, leukotrienes, prostanoids, and NO. As well, we have chemokines, which are small peptides (8000 Daltons) that facilitate the passage of leukocytes from circulation into the tissues. The prototypical chemokine is the neutrophil chemoattractant IL-8, which also activates neutrophils to degranulate and cause tissue damage. Cytokines IL-1 and TNF are thought to have primary roles in the pathogenesis of many disease states. They can be induced by variety of mechanisms, such as infection, trauma, ischemia, immune-activated T cell, or toxins. They aggravate disease states by such mechanisms as inducing endothelial adhesion molecules, essential for the adhesion of leukocytes to the endothelial surface prior to emigration into the tissues (Chin et al., 1990). Taken together, these are the pro-inflammatory cascade of gene products usually not found in the healthy population and they initiate the cascade of inflammatory mediators by targeting the synovium and joints in RA, as well as other organs, such as the endothelium and the heart.

TNFα

TNFα, or cachectin, is a soluble 17 KDa protein existing as a trimer, produced mainly by monocytes/macrophages, but also by mast cells, B cells, T cells, and fibroblasts with any potential noxious stimuli (Vassalli., 1992). Newly synthesized TNFα is inserted into the cell membrane and subsequently released through the cleavage of its membrane-anchoring domain by a serine metalloproteinase (Black et al., 1997). *In vivo*, TNFα is the most rapidly produced pro-inflammatory cytokine, with serum levels detectable in mice within 30 min (Tracy et al., 1987). In fact, in many chronic inflammatory diseases, such as RA, pre- and post infarct patients, angina, and atherosclerosis, TNFα concentration is significantly increased and appears to be the dominant cytokine (Maury., 1989; Lepore et al., 1994; Mutru et al., 1985). They exert their action by binding to specific high affinity cell-surface receptors and initiating a series of intracellular signal transduction pathways to stimulate early response genes (Kuby., 1997). Therefore, blocking TNFα will have a local, as well as a global effect on inflammation as it has a dominant role in promoting inflammation, through potent autocrine and paracrine induction of other inflammatory cytokines, including IL-1, IL-6, IL-8 and granulocyte-monocyte stimulating factor (Figure 1-2) (Nawroth et al., 1986; Butler et al., 1995; Haworth et al., 1991). TNFα also induces fever, either directly via stimulation of prostaglandin E_2 (PGE_2) synthesis by the vascular endothelin of the hypothalamus, or indirectly by IL-1 release (Warren., 1990). Results of studies in animals provide further evidence of importance of TNFα in RA. For example, transgenic mice expressing a deregulated human TNFα gene develop a spontaneous RA-like destructive polyarthritis (Keffer et al., 1991). Pretreatment of these animals with monoclonal

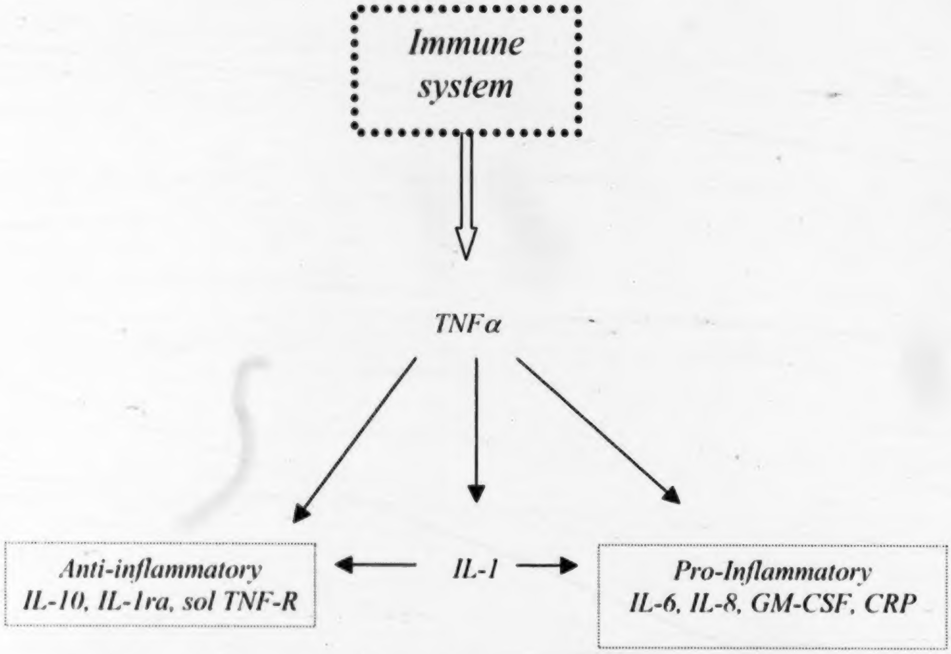


Figure 1-2: Cytokine cascade from the increasing TNF α levels in rheumatoid arthritis. Adapted from Feldman M, Elliot MJ, Woody JN, Maini RN. Anti-tumor necrosis factor-alpha therapy of rheumatoid arthritis. *Advances in Immunology* 1997; 64: 283-350.

antibody against TNFα prevents the development of arthritis. Several treatments, blocking TNFα binding and receptor activation, have been developed and are undergoing clinical trials. A soluble TNF receptor fusion protein made up of two recombinant p75 soluble TNF receptors fused with the Fc portion of human IgG1, called etanercept, is a dimeric structure 1000 times more efficient than a monomeric soluble p5TNF receptor at neutralizing TNF α (Mohler et al., 1993). Several placebo-controlled trials with patients having RA have indicated twice weekly subcutaneous injections of 25 mg etanercept results in significant improvement, with minor adverse effects (Moreland et al., 1997; 1999) Combination with methotrexate was significantly more effective than monotherapy in 89 patients with RA who have had a partial response to methotrexate (Weinblatt et al.,1999). Anti-TNFα therapy using chimeric monoclonal IgG1 antibodies against TNFα, called infliximab, is also an option for those exhibiting acute and chronic inflammatory state, such as RA, SLE (Feghali et al., 1997), and chron's disease (Kirman et al., 2004). A single intravenous dose of 10 mg of infliximab/kg rapidly reduces the number of swollen joints in RA as well as the serum concentration of CRP (Elliott et al., 1994). The tissue content of E-selectin and vascular cell adhesion molecule 1 in synovial biopsies also show significant reductions with infliximab treatment (Tak et al., 1996). It should be noted that TNF-receptor blockade is now the treatment of choice for RA (Yeh., 2004).

IL-1

Encoded by two different genes, IL-1 α and β are 17.5 kDa for the secreted molecule and they are secreted by the same sources as TNFα. The IL-1 signaling system appears to be more complex than the TNFα system, as it binds to two types of cell surface receptors. Only type I receptors have a cytoplasmic tail and are capable of intracellular signaling (Sims et al., 1993). Type II receptors are decoy receptors in which they bind circulating IL-1 but do not deliver any intracellular signals (Colotta et al., 1993). Soluble forms of both types of receptors, as well as naturally occurring antagonist (IL-1ra), compete with cell surface receptors, thereby decreasing IL-1 mediated activation of cells (Svenson et al., 1995). TNFα and IL-1 work synergistically to trigger fever, headache, myalgias, and arthralgias by PGE$_2$ synthesis by the vascular endothelium of the hypothalamus and stimulate T cell proliferation (Dinarello., 2000). IL-1 also elicits the release of histamine from mast cells at the site of inflammation, which, in turn, trigger early vasodilation and increase vascular permeability (Warren et al., 1990). IL-1 is strongly implicated in joint damage via stimulating the release of matrix metalloproteinases from fibroblasts and chondrocytes (MacNaul et al., 1990). As blocking the ability of a receptor to bind its cytokine is a useful strategy in signal interruption, a recombinant human IL-1 receptor antagonist has been studied clinically, with moderate clinical improvement (Jiang et al., 1998). However, the drawback to the therapeutic use of IL-1r antagonist is the short $t_{1/2}$ (6 h) in plasma, thus necessitating frequent daily treatment (Campion et al., 1996).

IL-6

A pleiotropic inflammatory cytokine produced by T-cells, monocytes, macrophages and synovial fibroblasts, IL-6 was identified as a factor inducing the final maturation of B cells into plasma cells (Van Snick., 1990). IL-6 function is ambiguous, as it appears to be both an anti-inflammatory, as well as a pro-inflammatory mediator. Its anti-inflammatory properties include the induction of the release of IL-1ra, as well as inhibiting the release of IL-1 and TNF (Gabay et al., 1997; Schindler et al., 1990). However, IL-6 involvement has also been clearly implicated in propagating RA-accelerated insulin resistance, atherogenesis, as well as bone resoption, muscle atrophy and anemia. Further inflammatory induction is observed in its production of plasminogen activating factor (PAF) and superoxide anions through neutrophil priming (Biffl et al., 1996; Borish et al., 1989). Deleterious activity of IL-6 *in vivo* has been suggested by experimental models of ischemia reperfusion and lung injury (Cuzzocrea et al., 1999a, b). Presently, a clinical trial of a humanized monoclonal antibody against the IL-6 receptor is currently under way for the treatment of RA (Choy et al., 2001).

Interferon (IFN) α

Interferon (IFN)α, the first cytokine to be produced by recombinant DNA technology, has emerged as an important regulator of growth and differentiation. It affects cellular communication and signal transduction as well as immunological control. Since the discovery of IFN in the 1950s, there has been increase in application of IFN in clinical treatment including virology, cell biology and immunology (De Maeyer E and De Maeyer-Guignard J., 1988).

IFNα possesses both antiviral and antitumor activity. Patients with various neoplastic and chronic viral diseases have been treated with IFN with variable results. These conditions include chronic myeloid leukemia, hairy-cell leukemia, lymphoma, malignant melanoma, and carcinoid tumors, as well as chronic hepatitis B and C and human related Kaposi sarcoma (Talpaz et al., 1986; Foon et al., 1986; Leavitt et al., 1987;

Vugrin et al., 1985; Borgstrom et al., 1982; Quesada et al., 1986; Oberg et al., 1986., Gutterman., 1994).

Like most other cytokines, the interferons are produced by the body, and they act locally at the site of inflammation. Systemically, certain toxic effects are seen, such as skin, neurologic, endocrine and immune toxicities. While rarely associated with toxicity, neutralizing antibodies have developed in patients treated with certain forms of IFN α (Gutterman et al., 1982; Antonelli et al., 1991; Bocci., 1991). The antibodies may abrogate biological effects *in vivo*, and the amino acid composition might explain the difference in immunogenicity between recombinant subtypes (Gutterman., 1994).

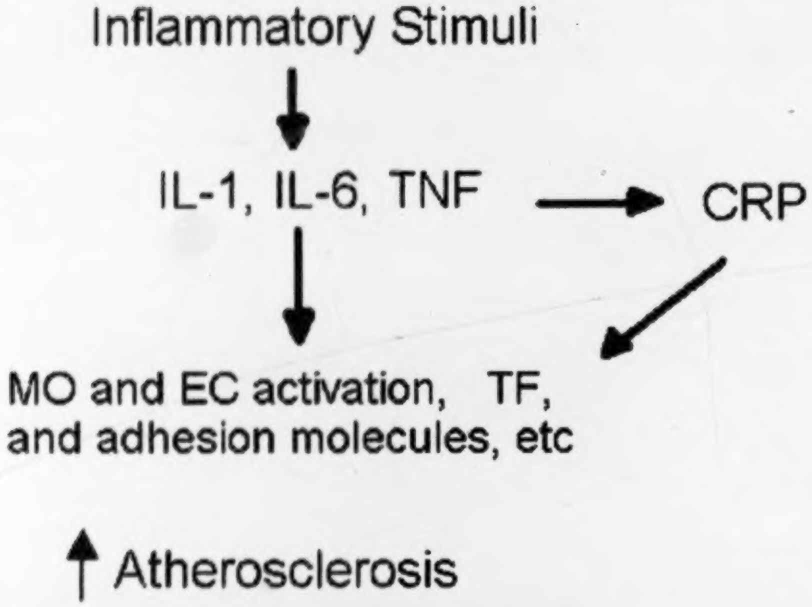
Interestingly, development of arthritis as a complication of $IFN_{\alpha 2a}$ treatment in individuals with various conditions has been observed (Nesher et al., 1998). $IFN_{\alpha 2a}$ administered to individuals with chronic hepatitis C caused depression in these patients, as well as a worsening of their arthritic conditions (Malaguarnera et al., 1998). Thus, a careful selection of hepatitis C patients is recommended before initiating $IFN_{\alpha 2a}$ therapy.

The effects of interferon on pre-existing arthritis may be explained by the fact that IFNs have multiple effects on the immune system. An enhancement of immunoglobulin production by B cells is observed at low IFN concentrations, whereas high IFN concentrations lead to its suppression (Pestka et al., 1987). Interferon also modulates the activity of natural killer cells and monocytes/macrophages and inhibits T-suppressor cell functions. In addition, enhanced expression of major histocompatibility complex class I and II antigens is observed with increased IFN levels (Pestka et al., 1987). Consequently, IFN will induce or worsen autoimmune diseases but also may induce remissions. This is reflected in the case of psoriasis, in which IFN therapy may results in either remission or exacerbation of the disease (Ziegler-Heitbrock HWL et al., 1989; Kurzrock et al., 1988). In fact, IFNα stimulates production of Th1 cells, which make IFN γ and IL-2, at the expense of Th2 cells, which make IL-4 andIL-5 (Parronchi et al., 1992). Elevated IFN levels are described in several autoimmune diseases (Pestka et al., 1987; Clarkson et al., 1993). In cases of SLE and rheumatoid arthritis, it may correlate with the disease activity. The involvement of IFNα as a potent inducer of Th1 is reflected on its role in aggravating pre-existing RA, as well as increasing the incidence of autoimmune disease between 4-19 % (Ioannou et al., 2000). IFN can induce production of a lupus idiotype *in vitro*. Indeed, IFN therapy is associated with the production of various autoantibodies, including antibodies to thyroglobulin, microsomal thyroid antigen, nuclear antigens and epithelial cells. (Pestka et al., 1987; Sen et al., 1992; Clark., 1991)

Cytokine Stimulated Inflammatory Mediators

C-Reactive Protein

In response to most forms of inflammation, there is the synthesis of a number of acute-phase proteins from the site of pathology, but principally in hepatocytes, under the control of IL-1, TNF α and IL-6 (Gabay et al., 1999). C-reactive protein was the first acute phase protein to be described over 70 years ago to be a blood protein that binds to the C-polysaccharide of pneumoccocci (Yeh., 2004; Hirschfield et al., 2003). CRP is a pentamer of 23 kDa subunits. The level is usually low in normal individuals, and acceptable literature values vary, ranging from 0.3 mg/l to 0.8 mg/l. Following an acute-phase stimulus however, values may increase by as much as 10,000 fold. *De novo* hepatic synthesis of CRP starts very rapidly, with serum concentrations rising by about 6 h, and peaking around 48 h after a single stimulus (Kushner et al., 1978). CRP levels are high in RA patients, as well as many CV disorders such as acute myocardial infarction, ruptured plaques, and coronary syndromes (Sano et al., 2003). It is a useful non-specific biochemical marker of inflammation, as the plasma $t_{1/2}$ is about 19 h under all conditions and is sole determinant of the plasma concentrations. The synthesis rate, therefore, reflects the intensity of the pathological process (es) stimulating CRP production (Hirschfield et al., 2003). Human CRP exerts its effects by binding with highest affinity to phosphocholine residues and extrinsic ligands, then precipitating or aggregating the cellular structures bearing these autologous or extrinsic ligands (Hirschfield et al., 2003). When human CRP is ligand-bound, it is recognized by C1q and potentially activates the complement pathway, engaging C3, the main adhesion molecule of the complement system, and the terminal membrane attack complex C5-C9 (Mold et al., 1999; Volanakis et al., 1974; Siegel et al., 1974). The secendary effects of CRP that follow ligand binding resemble key properties of antibodies, suggesting possible CRP contribution to host defense against infection and role as a pro-inflammatory mediator (Figure 1-3) (Hirschfield et al., 2003). High levels of CRP are a reliable predictor of outcome in patients with CV disorders, and are useful in the prediction of future cardiovascular risk in apparently healthy men and women (Ridker et al., 2001; 1997). In CV diseases, circulating CRP binds selectively to oxidized and enzyme-modified low density lipoproteins (LDL) as found in atheromatous plaques, and is deposited in majority of such plaques (de Beer et al., 1982; Zhang et al., 1999). The range of CRP's pro-inflammatory properties may



CRP indicates C-reactive protein; EC, endothelial cell; IL-1, interleukin-1; IL-6, interleukin-6, MO, monocyte-derived macrophage; TF, tissue factor, and TNF, tumor necrosis factor.

Figure 1-3: Pathway of CRP production and effect as a pro-inflammatory mediator. Adapted from Yeh ETH: CRP as a mediator of disease. *Circulation* 2004; 109 [suppl II]: II-11-II-14

include tissue damage, coronary calcification and insulin resistance (Hirschfield et al., 2003). More substantial CRP values are associated with poor prognosis in severe unstable angina (Liuzzo et al., 1994), future stroke, as well as outcome following stroke (Di Napoli et al., 2001) and the outcome in chronic renal disease (Arici et al., 2001). CRP level elevation is also observed in CV disorders in non-postoperative arrhythmia patients (Chung et al., 2001). The Prevention of REnal and Vascular ENdstage Disease (PREVEND) study also indicated the strong association between ECG abnormalities, measuring the T-axis, which is a strong independent predictor for fatal and nonfatal cardiac events, and CRP (Asselbergs et al., 2003). Taken altogether CRP involvement in inflammatory disorders appears to be becoming prominent, thus warranting further investigation of the effect of lowering CRP levels in improving mortality in CV patients. Indeed, large clinical studies indicate CRP level lowering with statins, independent of lipid lowering effect of statins, may have beneficial effects in survival rates (Albert et al., 2001).

Inducible Nitric Oxide Synthase

The diverse biological actions of NO have been defined in biological systems depending on the enzymic which it is produced from, the nature of stimulus, the amount produced and the site of secretion. NO acts as a mediator of vasodilation, platelet aggregation, neurotransmission, regulator of death and survival of various cell types (Moncada et al., 1991; Lincoln et al., 1997; Coleman et al., 2002). In general, in physiological concentrations, NO generated is in the picomolar range, and is constitutively produced by calmodulin-dependent NOSs (endothelial and neuronal NOS) (Moncada et al., 1991; Lincoln et al., 1997). NO acts directly on guanylyl cyclase to induce cGMP production, leading to controlled vasodilation. In contrast, however, immune and inflammatory responses via many cell types such as monocytes/macrophages, fibroblasts, and NK cells induce NO production via iNOS, leading to the production of relatively slow, sustained and high levels of NO (Moncada et al., 1991; Lincoln et al., 1997; Nathan et al., 1994). The over-expression of NO leads to the production of reactive nitrogen oxide species (RNOS) from NO_x, generated from oxygen. As RNOS are unstable, they rapidly S-nitrosylate cellular thiols including amino acids cysteine and glutathione, key targets in cell regulation (Butler et al., 1995; Wink et al., 1996). Oxidative stress through NO interaction with the superoxide anion (O_2^-) generates the highly reactive peroxynitrite anion ($ONOO^-$) (Wink et al., 1996). Increase in NO in synovial joints has been implicated in RA pathogenesis (Sattar et al., 2003), possibly inducing further pro-inflammatory cytokine production, as well as cell proliferation and differentiation (Schwentker et al., 2002). Inducible NOS knock-out animals or iNOS inhibitors have shown to ameliorate the physiological damage caused by excess NO produced with inflammatory stimulus (Coleman et al., 2002).

Inflammatory actions of Angiotensin II (AGII)

During the last few years, an exponential rise has been seen in the understanding of function and signal transduction mechanisms for AGII via the AT_1R and AT_2R. The active component of the renin-angiotensin system (RAS), this octapeptide hormone is the product of the inactive decapeptide angiotensin I (AGI), formed from tissue angiotensinogen by renin. AGI is then converted by angiotensin converting enzyme (ACE) to form angiotensin II (AGII), which has traditionally been seen to maintain blood pressure, fluid and electrolyte homeostasis and aldosterone release (Ruiz-Ortega et al., 2001). AGII is formed within the blood as well as local interstitium of tissues (Thomas., 1999). In particular, the role of AGII is mostly mediated through its binding to the high affinity, G-protein coupled angiotensin II receptor, type I and type II (AT_1R, AT_2R).

Molecular Biology of AT_1R and AT_2R

The use of selective antagonists has revealed the heterogeneity of AT-Rs and different cDNAs corresponding to each receptor (Inagami., 1999), allowing the two structurally similar receptors, with distinct pathways to have opposite physiologic effects (Figure 1-4). The vascular actions of AGII however have been attributed to the stimulation of the AT_1R subtype. The 7-transmembrane G-protein coupled receptors (GPCR) are expressed on many tissues and organs, including liver, heart, brain, gut, adrenal kidney, endothelial cells, monocytes and vascular smooth muscle cells (VSMCs) (Sayeski et al., 1998; Thomas et al., 1999). AT_1R activation induces a cascade of cellular responses, including rapid activation of phospholipase C β1 (PLC β1) dependent activation of protein kinase C (PKC) via Gq/11 heterotrimeric G proteins and intracellular calcium release (Brasier et al., 2002; Thomas et al., 1999; Dostal et al., 1997). AT_1R stimulation by AGII also activates intracellular signaling pathways traditionally associated with growth factor and cytokine receptors through tyrosine kinase stimulating pathways. They include tyrosine phosphorylation, and activation of PLCγ1, mitogen activated protein (MAP) kinases, and the JAK/STAT pathway (Marrero et al., 1995; Berk et al., 1997; Dostal et al., 1997). The processes that couple AT_1R activation to these diverse, growth-factor like signaling pathways are not known, but may involve the

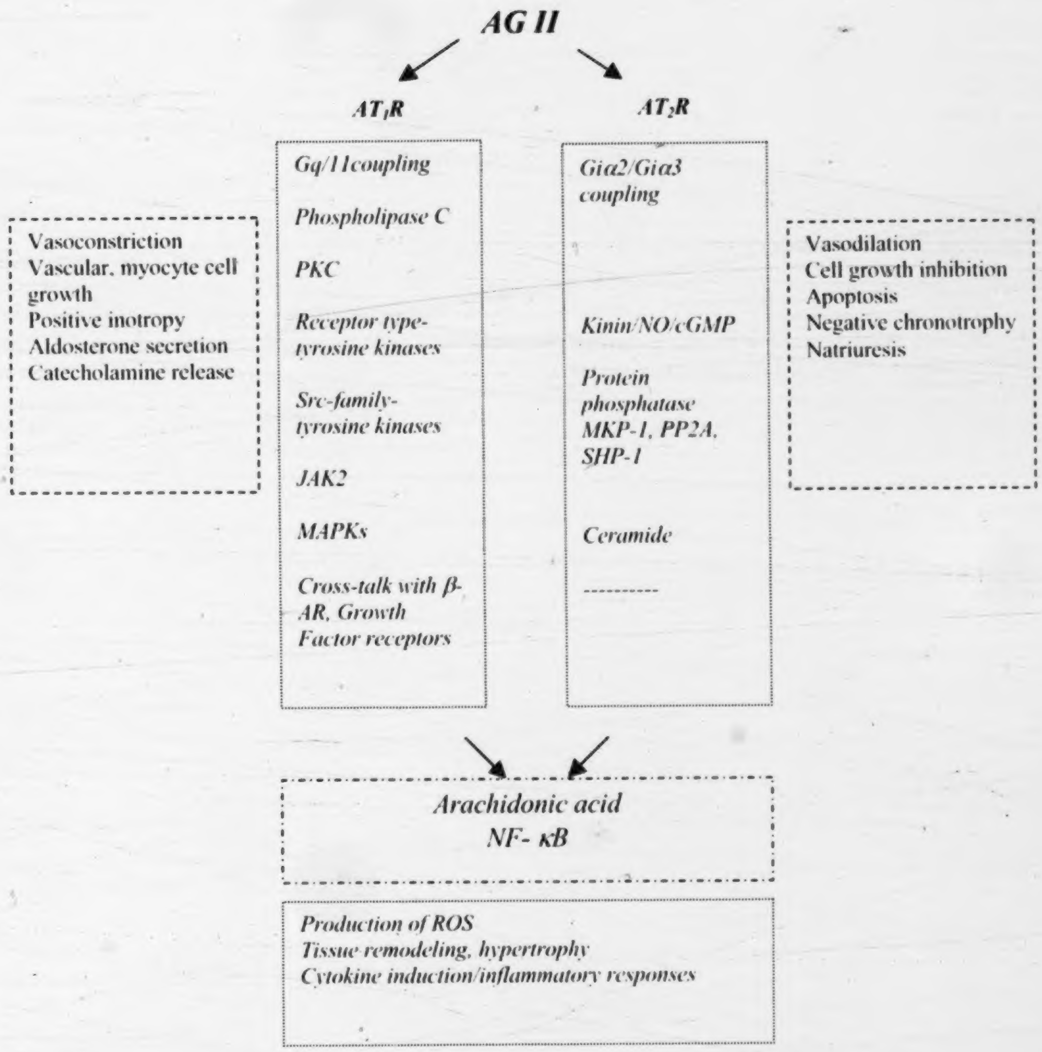


Figure 1-4: AGII mediated effects via stimulation of AT_1R and AT_2R

"classical" G-protein activation, or may be through non-conventional interaction and activation of signaling molecules other than the G-proteins coupling with the 7-transmembrane AT_1R (Thomas 1999). Ultimately, AGII can elicit trans-activation of growth factor receptors upon AT_1R stimulation, and thus regulate fibronectin and pro-inflammatory actions (Moriguchi et al., 1999). AT_2R development is primarily highest during fetal development, but drops after birth. AT_2R expression is also modulated by multiple growth factors, various other cytokines, as well as through self-induction by AGII (Horiuchi et al., 1999). AT_2R stimulation activates NO production through cGMP, as well as phosphatase(s) resulting in the inhibition of ERK and Bcl-2 dephosphorylation. Moreover, AT_2R stimulation increases ceramide production, leading, overall, to apoptosis and antigrowth effects (Figure 1-4). Interestingly, increasing vascular diseases, injury and CVD appear to be marked also by increased expression of AT_2R, which is up-regulated along with AT_1R expression (Peng et al., 2002; Horiuchi et al., 1999). It appears that up-regulation of the AT-Rs may be mediated by the increasing pro-inflammatory cytokine production observed in these disease states. Increasing IL-6 and TNFα appear to be some of the instigators in the up-regulation of the AT_1R in endothelial dysfunction and cardiac cells (Peng et al., 2002; Wassmann et al., 2004). Vascular remodeling and apoptosis, as seen in atherosclerosis and angina, are attributed to AT_2R up-regulation through increasing inflammatory mediators (Akishita et al., 2000). Up-regulation of AT-Rs would potentiate the effect of AGII in disease states. Moreover, increases in AGII activation mark CVDs such as hypertension, MI and atherosclerosis (Virdis et al., 2003). By the same token, markers of inflammation have been shown to be up-regulated in different forms of cardiovascular disease, correlating with the vascular risk. Increasing activity of AGII, as a pro-inflammatory mediator, is evident in the increase in ROS, adhesion molecules, chemokines and cytokines (Ruiz-Ortega et al., 2001; Virdis et al., 2003). Increased level of selectins and adhesion molecules have been observed in hypertensive patients as well as patients with coronary artery disease. Increased production of P-selectin, and the corresponding increase in angiotensin II induction of the adhesion of monocytes and neutrophils to endothelial cells, is implicated (Prasad et al., 2001). Increased monocyte/macrophage infiltrate, as well as ICAM-1 up-regulation, is also observed in perivascular space in cardiac tissue and VSM (Muller et al., 2000). The up-regulation in activity of nuclear factor κB (NFκB) through AGII activation of AT-Rs is thought to promote and accelerate the inflammatory atherosclerotic process and cardiovascular inflammatory injury (Ruiz-Ortega et al., 2000; Virdis et al., 2003; Sadoshima., 2000). More and more reports define the cytokine-like actions of AGII and its role in activating cells regulating the expression of many substances, such as growth factors, cytokines, and chemokines. The vascular response to AGII is further up-regulated through the up-regulation of AT_1R, as seen in studies with AT_2 knockout mice (Tanaka et al., 1999). It appears that cytokines and pro-inflammatory mediators such as CRP up-regulate the AT_1R in end-stage heart failure, VSM, and in experimental myocardial infarction (Kaprielian et al., 1997; Wang et al., 1999; de Boer at al., 2003). Cardiac hypertrophy is also defined by an over-expression of the AT-Rs, especially AT_1R, as AGII also acts as a growth factor, inducing hyperplasia/hypertrophy depending on the cell type (Egido., 1996). Vascular dysfunction and increasing pro-inflammatory levels eventually lead to the varying cardiovascular disorders such as hypertension, atherosclerosis, infarction, left ventricular hypertrophy, and heart failure.

Therefore, the control of AGII effect and thus the control of inflammatory states, either directly or indirectly, become important. Presently, the use of ACE inhibitors and AT_1R antagonists is more prevalent in the treatment of not only hypertension, but also varying cardiovascular diseases. Numerous studies indicate renin angiotensin system inhibitors lower AGII levels and activity and pro-inflammatory cytokine levels in disease states. The clinical outcome of ACEI and AT_1R antagonists, which appear to ameliorate the inflammatory cascade, is of great importance, considering the prevalence of various inflammatory disease states with an underlying cardiovascular complication.

ACE and AT_1R Inhibition: Therapeutic Relevance

It appears that ACEI and AT_1R antagonists modulate the balance between pro- and anti-inflammatory cytokine productions and are thus potentially useful as therapeutic agents. *In vitro* reports find valsartan and captopril to be effective agents against lipopolysaccharide (LPS) induced stimulation of TNFα and IL-1 at high doses, while increasing the anti-inflammatory cytokine IL-1Ra (Peeters et al., 1998). However, single dose treatment in essential hypertensive patients does not influence LPS-stimulated production of cytokines by whole blood (Peeters et al., 1998). Interestingly, AT_2R stimulation may compensate, and may in fact counteract, the inflammatory effects induced by AT_1R stimulation. This is suggested by the decreased effectiveness of AT_1R antagonist valsartan in AT_2 null nice compared with controls (Wu et al., 2001).

In vivo experiments and clinical studies in inflammatory processes and cardiovascular disorders also indicate a decrease in elevated levels of pro-inflammatory cytokines with the treatment of ACEI and AT_1R antagonists. In myocardial infarcts, administration of losartan or captopril decreased the TNFα mRNA expression, which had increased with AGII administration (Frolkis et al., 2001). One dose of enalapril was enough to decrease the mRNA expression of TNFα and IL-6 in endotoxin-induced cytokine production in SHR (Niimi et al., 2002). In cardiac heart failure, captopril also dose dependently suppressed IL-1 and TNFα production by human mononuclear cells by 60 and 74 % respectively in patients with heart failure (Schindler et al., 1995). Chronic treatment with captopril, enalapril, fosinopril or ramipril also induced cardiac regression in LVH and decreased ACE activity (Raasch et al., 2002). In chronic heart failure, 14-week treatment with candesartan decreases plasma levels of TNFα, IL-6 and various adhesion molecules (Tsutamoto et al., 2000). Yet another study with patients with premature atherosclerosis, irbesartan significantly reduced levels of adhesion molecules VCAM-1, soluble TNFα, and superoxide levels, with the maximal effect on cardiac artery disease seen at 12 weeks (Navalkar et al., 2001).

Expression of other inflammatory mediators, such as inducible nitric oxide synthase (iNOS), is also decreased with the use of ACEI such as quinapril (Bachetti et al., 2001). The NO_2-NO_3 and TNFα levels, elevated during the LPS induced inflammation in SHR, were also significantly attenuated.

The beneficial effects of AT_1R antagonists and ACEI in cardiovascular diseases can be extended to other inflammatory conditions with elevated cytokine levels. In fact, any organ in which the AT_1R is expressed most likely will benefit from RAS inhibitors, as increasing cytokine levels will, in the long run, increase cardiovascular pathogenesis and compromise organ function. Thus, the ability of the body to respond to therapy will be further compromised in hyper-inflammatory conditions, whether it is pharmacokinetic or pharmacodynamic in nature. We therefore decided to investigate if AT_1R antagonists will be affected in a model of inflammation, as seen in rheumatoid arthritis. Losartan and valsartan were chosen for their varying pharmacokinetic and pharmacodynamic properties.

Losartan Pharmacokinetics and Pharmacodynamics

Losartan is the first of the series of non-peptide, orally active selective angiotensin II type 1 receptor antagonist, containing the Takeda series of 1-benzyl imidazole-5-acetic acid derivatives, with a biphenyltetrazole moiety (Lo et al., 1995; McIntyre et al., 1997). The oral bioavailability of losartan tablet is less than 30%, with the peak concentration attained within 30 to 60 min (McIntyre et al., 1997). Losartan is the only other AT_1R antagonist, aside from candesartan cilexetil, which undergoes extensive first-pass metabolism in the liver. Only 1/3 rd of the oral dose reaches the systemic circulation unchanged. *In vitro* studies have revealed the metabolism and clearance of losartan follows one of two major pathways and involves the oxidation of the benzylic imidazole substitutents by CYP 450 in liver cells, which is followed or preceded by glucoronidation (Stearns et al., 1995). At least five metabolites of losartan have been identified and are presented in Figure 1-5 (McIntyre et al., 1997; Schmidt et al. 2003). The oxidation of the alcohol side chain of losartan into the carboxylic acid moiety in the major pathway gives the aldehyde EXP 3179, which is rapidly oxidized to the active metabolite EXP 3174. The oxidation is mainly performed by hepatic CYP 2C9 and 3A4 enzymes, with only 14 % of the total losartan dose being converted to EXP 3174. However, the resulting EXP 3174 is 10-40 times more potent as an AT_1R antagonist than losartan, and is responsible for most of the receptor antagonism seen with losartan

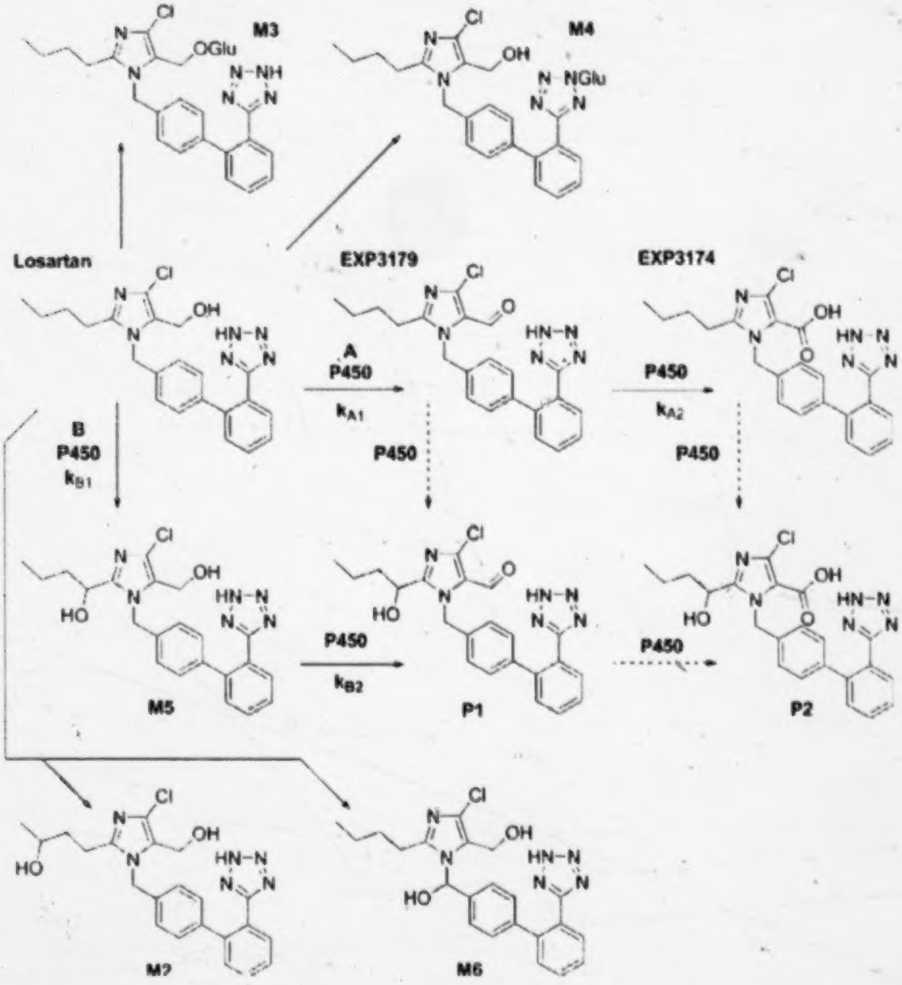


Figure 1-5: CYP 450 oxidation of losartan. Obtained from Stearns RA, Chakravarty PK, Chen R, Chiu SHL. Biotransformation of losartan to its active carboxylic acid metabolite in human liver microsomes. *Drug Metab Dispos* 1995; 23: 207-215.

(Kaukonen et al., 1998; Lo et al., 1995; Yasar et al., 2002; Yun et al., 1995; Schmidt et al., 2003). Approximately 5% of the dose is excreted unchanged in the urine and about 8% of the dose is excreted in the urine as EXP 3174. In total, ~ 92% of the administered dose can be accounted for by excretion of drug in urine and feces (McIntyre et al., 1997). As well, binding studies indicate losartan antagonism of the AT_1R is competitive with increasing AGII, while EXP 3174 exhibits noncompetitive antagonism (See., 2001). The measured and predicted bioavailability from the hepatic extraction ratio show absorption of losartan from the gastro-intestinal tract (GIT) is complete. Losartan, but not EXP 3174, is also a substrate for P-glycoprotein (Pgp) (Soldner et al., 2000). Both losartan and EXP 3174 are highly protein bound to albumin (98.7% for losartan, 99.8% for EXP 3174) with the final Vd being 34 L for losartan and ~ 10 L for EXP 3174 (Christ., 1995). Neither losartan nor Exp 3174 are significantly displaced *in vitro* by therapeutic concentrations of highly protein bound drugs such as naproxen, ibuprofen, diazepam, or warfarin, suggesting clinically significant drug interactions are unlikely to occur (Christ., 1995). Disease states mainly appear to alter the active metabolite levels rather than the parent drug. In elderly patients, single oral and *iv* doses of losartan did not alter its concentrations, while EXP 3174 levels were increased 3 fold. The efficacy and safety however remained unaltered (McIntyre et al., 1997). Liver disease also appeared to increase the EXP 3174 two- fold as well, and drug level monitoring appear to be recommended (Christ et al., 1995). Renal insufficiency, as seen in moderate to severe kidney disease (Clcr of 10-29 ml/min) decrease the renal clearance of losartan and EXP3174, although the levels of EXP 3174 was unchanged (Sica et al., 1995).

Valsartan pharmacokinetics and pharmacodynamics

Valsartan is the S-enantiomer of N-valeryl-N-[[2'-(1H-tetrazol-5-yl) biphenyl-4-yl] methyl]-valine (Figure 1-6), as the R-enantiomer has 170-fold less activity in terms of AT_1R binding. Valsartan is a competitive antagonist against AT_1R with 20,000 fold higher potency for AT_1R than for AT_2R (See., 2001). At least 51 % of the dose is absorbed, with ~ 92 % of the drug being bound to plasma albumin, and ~22% binding to AAG. The Vd is therefore small, at 16.91 ± 6.90 L (Thürnmann et al., 2000). The accumulation factor is small with multiple dosing at 1.21 with 200 mg o.d over 8 days. No change in antihypertensive effect of valsartan is observed however. Waldmeier et al administered ^{14}C-labeled valsartan to six healthy volunteers to observe the excretion pattern. Ninety six percent of ^{14}C-labeled valsartan was recovered from plasma, feces, and urine. Biliary excretion was found to be the primary elimination route of valsartan, with ~ 70 % of the dose excreting unchanged through the biliary route. The only verified metabolite was V-M1 (Figure 1-6) was identified by MS and ^1H NMR and confirmed by total synthesis (Waldmeier et al., 1997) and metabolism contributes to less than 20 % of its clearance. A significant food dependency of the bioavailability is unique to valsartan and may result in up to 50 % reduction in drug availability. Valsartan is not recommended for patients with severe hepatic dysfunction and/or biliary cirrhosis (Brookman et al., 1997; Martin et al., 2002). Dose adjustment should also be considered in 70-year old patients, because plasma clearance of valsartan is predicted to be 22 % lower compared with that in an average 55 year old (Sioufi et al., 1998). As well, those

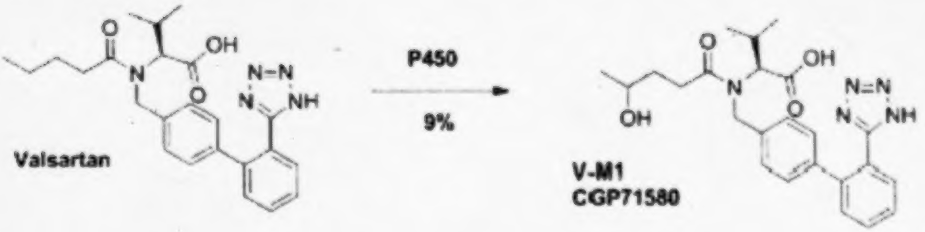


Figure 1-6: Structure and metabolic pathway of valsartan. Adapted from Schmidt B, Schieffer B. Angiotensin II AT1 Receptor Antagonists. Clinical Implications of Active Metabolites. *J Med Chem* 2003; 46: 2261-2271.

with severe renal impairment should be considered for dose reduction of valsartan, although as the fraction of valsartan excreted unchanged by the kidney is only 10-15%, dose reductions in mild-to-moderate renal impairment or the elderly do not routinely have to be considered (Martin et al., 2002). Both losartan and valsartan lack affinity for adrenergic, histamine, substance P, muscarinic and serotonin receptors (Burnier., 2001)

Clinical Applications of Losartan and Valsartan

AT_1R antagonists are considered as an alternative therapy for essential hypertensive patients intolerant of ACEI due to the persistent cough caused by increase in bradykinin levels. As well, AT_1R antagonists will decrease the activity of AGII produced through alternate pathways from the ACE. The deleterious effects of AGII on the heart and kidney in patients having high levels of plasma renin activity may then be ameliorated, thus decreasing the risk of stroke and MI (Burnier., 2001). Presently, in addition to the treatment of hypertension, losartan decreases mortality and morbidity as compared to some conventionally used β-blockers, such as atenolol (Okin et al., 2003, Lindholm et al., 2002). A similar reduction in blood pressure is also observed with AT_1R antagonists as with the β-blockers, however, with a better tolerability profile in some complications (Dahlöf et al., 2002). Indeed, losartan therapy has also been indicated, and its use approved, in patients with diabetic nephropathy, with proteinuria and hypertension, as the incidence of doubling of the serum creatinine concentrations and end-stage renal disease is reduced with treatment. It is also the only agent to demonstrate a significant delay in end-stage renal disease (Brenner et al., 2001). AT_1R antagonists, along with ACEIs, have been deemed beneficial in reducing complications associated with myocarditis by down-regulating the potential autoimmune component of the disease (Godsel et al., 2003). Interestingly, clinical trials show valsartan significantly reduces the combined end point of mortality and morbidity improving ejection fraction. The signs and symptoms of heart failure, as well as the quality of life improve significantly when compared with placebo when valsartan is added to prescribed therapy (Cohn et al., 2001). Losartan outcome is superior to atenolol in treating isolated systolic hypertension and left ventricular hypertrophy (LVH) (Kjeldsen et al., 2002).

With losartan in particular, recent investigations show novel AT_1R independent functions, which encompass anti-inflammatory and anti-aggregatory actions. The effects are not related to the anti-hypertensive effects, and are mediated through actions of another losartan metabolite EXP 3179 (Sadoshima., 2002). After single oral 100 mg losartan, inhibition of $PGF_{2\alpha}$ production was observed over 6-8 h *in vivo* corresponding to increasing EXP 3179 production (Krämer et al., 2002). It appears EXP 3179 is structurally similar to indomethacin, and therefore acting like a COX inhibitor. EXP 3179 may also share COX-like inhibition of NF κB, AP-1 or CCAAT enhancer-binding proteins, thus negatively regulating transcription of pro-inflammatory cytokines. As well, EXP 3179 acts as a receptor antagonist for TXA_2 receptors to prevent U46619-induced platelet aggregation (Sadoshima., 2002). In heart failure, MI and atherosclerosis, inflammatory mediators, as well as inflammatory receptors such as COX-2, PGE_2 and TXA_2, are up-regulated. AT_1R antagonists such as losartan and valsartan may therefore be much more preferable alternatives to other CV drugs in the treatment of CVD due to their added anti-inflammatory properties. Recent studies compared valsartan, simvastatin, quinapril on their suppressive action of ROS generation, NF κB production in mononuclear cells, and CRP levels. Valsartan, at 160 mg, exerted a profound and rapid ROS and inflammation-suppressive effect as compared to the other 2 treatments over a 1 week period (Dandona et al., 2003). The potential implications of the beneficial effects of valsartan in atherosclerosis, diabetes and CHF must be further investigated. The use of AT_1R antagonists are becoming more relevant and essential in the treatment of inflammatory and cardiovascular disorders, conditions which express increased circulating AGII levels and AT_1R up-regulation in cardiovascular disorders.

Influences from Pro-Inflammatory Mediators on Drug Pharmacokinetics

Absorption

Drug bioavailability from the GIT can be affected by alteration to the epithelial permeability, changes to the mucosal blood flow, varied expression of major drug metabolizing enzymes, such as CYP 3A4, and altered expression of P-glycoprotein (Pgp) (Bertilsson et al., 2001). CYP 3A4 accounts for ~30 % of hepatic CYPs, and >70 % of small intestinal CYPs. The highest concentration of CYP 3A4 is found just below the brush border, near the absorptive surface (Watkins., 1997). Recently, much investigation has focused on the expression of the multi-drug resistance (MDR) transporters, as well as the CYP enzymes, during inflammation. P-glycoprotein is best characterized as plasma membrane MDR protein. As they are efflux proteins, responsible for the active efflux of drugs and xenobiotics, they are thought to play an important role in systemic drug absorption. Substrates of Pgp are generally hydrophobic and structurally unrelated. In the GIT system, they are transported from gut epithelial cells and plasma back into the gut lumen, thus decreasing their bioavailability (Benet et al., 1996; Ayrton et al., 2001). Substrates dependent on gut CYP 3A4 enzymes and are Pgp substrates are thought to have limited exposure to the enzymes in the gut. The oral clearance of Pgp substrates can be related to the level of available gut Pgp. For example, cyclosporin oral clearance is positively correlated with Pgp levels, in which the higher the intestinal Pgp level, the higher the oral clearance (Hall et al., 1999). Substrates for Pgp include vinblastine, cyclosporine, verapamil, dihydropyridines, nicardipine, and nifedipine. Drugs and xenobiotics, which are Pgp substrates, can also act as Pgp protein inhibitors, and thus increase the absorption kinetics of other Pgp-dependent substrates.

Few studies have addressed the impact of inflammatory mediators on drug absorption and metabolism in intestinal cells, especially on the permeability and the expression of Pgp and CYP 3A4. However, results appear conflicting, possibly due to the varying cell lines and inflammatory process under investigation (Bertilsson et al., 2002; Kalitsky-Szirtes et al., 2004). Caco-2 cells express Pgp and when treated with 1α, 25-$(OH)_2D_3$ allow CYP 3A4 mRNA expression as well. The presence of five different pro-inflammatory cytokines, as released from co-cultured macrophages or added separately, diminished the expression of CYP 3A4 mRNA in all treatments compared to the untreated controls (Bertilsson et al., 2002). Results are in agreement with findings by Abdel-Razzak et al who saw a down-regulatory effect on CYP 3A4 expression in hepatocytes with cytokine treatment (Abdel-Razzak et al., 1993). Bertilsson et al however found a positive effect on MDR1 mRNA expression in epithelial cells with local inflammatory process, indicating the possibility of increased efflux action of Pgp substrates during inflammation. On the other hand, there was an increase in epithelial permeability of Caco-2 cells, possibly negating any decrease in absorption that may be observed with Pgp expression. Interestingly recent study by Kalitsky-Szirtes et al indicate that Pgp expression in the jejunum of rats treated with endotoxin or LPS is down-regulated by ~50% (Kalitsky-Szirtes et al., 2004). Corresponding reductions in the basolateral to apical efflux of digoxin, amiodarone, and carboxyfluorescein were observed, resulting in significant increases in the apical to basolateral absorption of these compounds (Kalitsky-Szirtes et al., 2004). Altogether, it appears that during inflammation, absorption of lipophilic drugs, which may also be a Pgp and 3A4 substrates, may be increased during inflammation, leading to increasing plasma levels of such drugs.

Hepatic Excretion and Transport Proteins

The role of transport proteins extends to the hepato-biliary excretion by the transfer of drugs to metabolic sites and /or the biliary canalicular membrane mediated by intracellular transfer proteins and passive diffusion. (Ayrton et al., 2001; Pascaud et al., 1998). The carrier proteins involved in the active excretion of drugs from the hepatocytes to the bile are the organic anion transporting polypeptide (OATP) and the ATP-dependent transport systems canalicular mutispecific organic anion transporter (cMOAT) and MDR, which includes the Pgp (Ayrton et al., 2001). Acidic, basic, zwitterionic and neutral compounds are all potential substrates for OATP, and MDR2 have been shown to mediate the transport of endobiotics, anionic drugs and drug conjugates, including prevastatin and methotrexate (Yamazaki et al., 1997; Hooijberg et al., 1999). Although some transport proteins are specific to the liver, cMOAT and OAT transfer proteins, as well as Pgp also mediate renal active excretion. Very few studies have been performed on canalicular transport of drugs during inflammation, as with the MDRs in the intestine. However, some have shown down-regulation of Pgp activity and levels in rat hepatocytes with increasing cytokine levels such as IL-1 β and IL-6 (Sukhai et al., 2001). The effect of inflammation on other transport proteins in the liver and kidney is yet to be investigated. However, the potential effect of cytokines and inflammatory mediators in the biliary excretion of drugs and to drug influx into the hepatocytes to the CYP enzymes must be considered, as it may increase the plasma levels of those drugs in the systemic circulation.

Metabolism

Inflammatory stimuli and pro-inflammatory mediators change the activities and expression of various forms of cytochrome P450 in the liver, kidney, and brain. Many important drugs, whether they undergo high or low extraction, rely on hepatic metabolism by CYP450s for clearance from the circulation and pharmacological activation or conversely, inactivation. Therefore, factors that modulate the CYP450s have the potential to adversely or favorably affect the therapeutic effects of drugs. (Morgan et al., 1997).

A large number of infectious or inflammatory agents have been shown to depress CYP450 catalyzed drug metabolism in humans and in experimental animals. The first observation of CYP being modulated during a host defense response was made by Samaras and Dietz in 1953 when they reported that the actions of pentobarbital were greatly exaggerated when the reticuloendothelial system (RES) was stimulated by trypan blue (Samaras et al., 1953). In humans, the first indication of clinically important infection-evoked alteration in metabolism was the major outbreak of influenza in Seattle (USA) that affected ~50% of the pediatric population. Eleven asthmatic children developed a sudden decrease in theophylline clearance and were hospitalized with toxicities ranging from headache to seizures (Kraemer et al., 1982). Patients with arthritis also exhibit a compromise in drug biotransformation (Ishizuki et al., 1983), and the loss of biotransformation has been linked to increased IL-1 production. Morgan *et al* report selective CYP 450 downregulation *in vivo* and *in vitro* with different diseases and models of disease (Morgan et al., 1997). Reduced clearances of antipyrine, hexobarbital and theophylline were observed in human volunteers given low doses of LPS (Shedlofsky et al., 1994). Moreover, reduced clearance of the drugs correlated with the initial peak values of TNFα and interleukin 6. Cytokine production may be systemic, from monocyte/macrophages, or local, via modulation of the RES, which includes hepatic kupffer cells (Morgan., 1997). In a complex *in vivo* inflammatory response, many other factors may potentially affect CYP450 expression. For instance, actions of cytokines in the central nervous system affect the hypothalamo-pituitary axis (Bernton et al., 1987), resulting in altered secretion of glucocorticoids, thyroid hormones, and growth hormone, each of which regulates CYP 450 expression (Morgan et al., 1998). Direct action of toxins on the CYP450 system is also observed, as LPS can act directly on hepatocytes and down-regulate CYP450 expression (Ansher et al., 1992). Despite the fact that many CYP450s are suppressed during inflammation or infection, it can be discerned from the literature that some are unaffected and some are induced, such as CYP 4As (Sewer et al., 1997). Differential regulation of CYP enzymes depends on the particular stimulus and infection (parasitic versus bacterial), the type of cytokine being produced, the tissue being studied, as well as the animal model being used (Morgan., 1997). LPS stimulation, for example, will release systemic IFNγ, TNFα, IL-1 and IL-6, and induce the local release of acute phase proteins from the liver. Cultured primary hepatocytes initially have high levels of total CYP 450 expression, which plummet during the first 24-48 h after inflammatory stimulation (Kocarek et al., 1993). However, there may not be a total change in CYP microsome level, as there is diversity in how individual CYP enzymes respond to stimulus. In fact, some enzymes are up-regulated while others are down-regulated by an inflammatory stimulus. Selective down-regulation or up-regulation of many constitutive and inducible CYP 450s by cytokines are thought to be due to repression of gene transcription, changes in RNA and/or protein turnovers or direct decrease in P450 catalytic activity (Iber et al., 1999; Jover et al., 2002.; Khatsenko et al., 1998; Shedlofsky et al., 1994). The pre-translational mechanism in the loss of the CYP isoform preceed the depression of specific mRNA species, and subsequent decrease in enzyme synthesis and activity is observed. Varying molecular mechanisms are involved in CYP enzyme down-regulation. The down-regulation of CYP 2C11 by IL-1 and IL-6 involves the proximal promoter region of the CYP2C11 gene (Chen et al., 1995). CYP1A1 regulation by IL-6 on the other hand appears to involve the induction of heme oxygenase (Fukuda et al., 1994). Increases in NFκB induction by IL-1 β may also be involved in changes to CYP gene transcription such as CYP2C11 (Iber .,2000) and rapid turnover of mRNA is shown to be accelerated by IFN in the rat (Delaporte et al., 1997). When activated, a latent endonuclease that cleaves single-stranded mRNA after UA, UG, UC, and UU residues at the 3' end could degrade mRNAs coding for CYP, accounting for the increased mRNA turnover (Salehzada et al., 1993). Protein degradations may also contribute to overall CYP loss, independently from the increase in mRNA turnover (Mannering et al., 1986).

The involvement of ROS and NO in CYP down-regulation have also been considered. Increasing oxidative stress observed in the liver during inflammatory response depresses CYP (Proulx et al., 1995), and the addition of anti-oxidants appear to prevent the loss in CYP and alterations in drug metabolism (Renton., 2001). Production of NO, possibly from iNOS stimulated during inflammation, is also speculated to be involved in CYP down-regulation. The magnitude of CYP down-regulation appears to correlate with NO increase. Nitric oxide appears to physically bind to enzyme protein, in particular to the prosthetic groups such as heme or iron-sulfur clusters, leading to activation or, more likely, the loss of CYP-mediated activity (Khatsenko., 1998). It is clear that the modulation in the CYP enzyme expression and activity during inflammation will alter the capacity to metabolize, activate, or de-activate drugs or endogenous materials.

Plasma Protein Levels

Highly protein bound drugs are at risk of altered distribution and elimination, because the concentration of plasma proteins change during inflammation. Alpha-acid glycoprotein binds to basic drugs, and is an acute phase protein, whose level increases markedly during systemic inflammation. On the other hand, plasma albumin, which binds mostly acidic xenobiotics, appears to be decreased during chronic inflammation. Albumin depression can be caused by increasing cytokine levels, as seen in patients with severe RA (Van Den Ouweland et al., 1988). Changes in protein binding will alter the clearance of drugs, as only the free drug can be metabolized and eliminated. The balance between bound and unbound drug may therefore be altered in inflamed states, for highly protein bound drugs (Mayo et al., 2000; Piquette-Miller et al., 1993).

The inflammatory response is not only limited to changes in the pharmacokinetics of drugs. Circulating cytokines alter function of various tissues, including adipose, skeletal muscle, liver, and vascular endothelium, to generate a spectrum of pro-atherogenic changes that will alter the tissue and organ function.

Influences of Pro-Inflammatory Mediators on Drug Pharmacodynamics

Studies have shown that β-adrenergic antagonists and calcium channel blockers have decreased potency in inflamed states (Guirguis et al., 2003; Kulmatycki et al., 2001; Mayo et al., 2000), which may be due to the effect of inflammatory mediators on the expression and function of G-protein coupled receptor (GPCR) and ion channels. Ion channel expression in disease state has not been extensively studied, although the ability of the ion passage into and out of the cells is often altered in disease states. Evidence has been accumulating on changes that possibly occur to G-protein function in cardiovascular disease states, which have also been expressing hyper-inflammatory activity, as discussed previously. The possible mechanism of down-regulatory effect on the GPCRs in disease states would, in fact, explain the therapeutic inefficacy being observed for cardiovascular drugs in inflamed states.

G Protein Coupled Receptor (GPCR) Activation

GPCRs constitute a superfamily of 7 transmembrane spanning proteins responding to a diverse array of sensory and chemical stimuli (Ferguson., 2001). GPCRs transduce the information into intracellular second messengers, which are then interpreted as meaningful signals by the cells. This process involves the coupling of agonist-activated GPCRs via interaction with heterotrimeric G-proteins. G-proteins consist of α, β and γ subunits (Lombardi et al., 2002). Binding of an agonist to a GPCR results in conformational changes in the receptor, which promote guanine nucleotide exchange (GDP for GTP) on Gα resulting in subsequent dissociation from the G$\beta\gamma$ subunits. The dissociated Gα GTP and $\beta\gamma$ subunits can independently activate or inhibit a range of signaling proteins including phospholipase C (PLC), adenylate cyclase (AC) and ion channels (Neer., 1995). Hence, the final outcome of most of the biological responses mediated by GPCRs is the result of a complicated network of intracellular signaling pathways. Changes seen in GPCR effectiveness in varying disease states may be due to any number of factors altering the signaling molecules.

GPCR Regulation: Implications in CVD and Inflammation

More and more studies in various cardiovascular and inflammatory diseases show that GPCR response, in particular the β-AR response, is impaired. Animal and human data in forms of ischemic heart failure, hypertrophic cardiomyopathy, hypertension, airway smooth muscle signaling and human bronchial epithelial cells indicate down-regulation in effect. For example, the positive inotropic effects of β_1-AR in heart and vasodilatory effects of β_2AR are diminished in the airway smooth muscle cells with inflammation (Ungerer et al., 1993, 1994; Penn et al., 1996; Hammond et al., 1992). Low-affinity agonist binding, decrease in adenylate cyclase (AC) activity, increase in phospholipase C (PLC) level and the inhibitory Gi proteins is also observed in diseases exhibiting hyper-inflammatory state (Lombardi et al., 2002; Hammond et al., 1992; Meij., 1996; Ungerer et al., 1994). When the G protein-coupled receptor kinases (GRK) and PKC expression and activity are also measured, an increase in expression of GRK2 (also known as βARK) and β-arrestin was observed in ischemic and failing heart, as well as hypertension (Gros et al., 1997,2000; Ungerer et al., 1994). GRK and β-arrestins are involved in GPCR desensitization process (Ferguson., 2001). As well, increasing catecholamine level and activity is also observed in varying inflammatory states and in CVDs such as heart failure (Meij., 1996; Egger et al., 1982; Harbuz et al., 1994). This, in turn, would increase receptor uncoupling, desensitization, and internalization, leading to the observed receptor down-regulation. In fact, the desensitization mechanism proposed with agonist-stimulation can either lead to receptor recycling and re-sensitization or be targeted to storage and/or lysosomes (Ferguson., 2001). This may lead to decrease in receptor density. The multi-faceted abilities of GRK and β-arrestins are also evident in immune cells, in which they are involved in mediating ERK activation and production of MAPK (Lombardi et al., 2002). It appears that PKA and PKC modulate GRK activity and membrane targeting, via transcriptional control at the mRNA level. Interestingly, GRK expression appears to decrease in inflammatory states such as rheumatoid arthritis, opposite what is observed in cardiovascular disorders. Chemokine and pro-inflammatory cytokines such as TNFα, IFN γ and IL-6 are increased in RA, and treatment of PBMC with these compounds strongly promotes GRK2 down-regulation (Lombardi et al., 1999, 2001). The down-regulation of GRK, in pre- and post-transcriptional regulation appears to be the instigators in GRK degradation and/or decrease in expression. In some cases, the GRK level is decreased but the β-arrestin levels are increased in varying inflammatory stimulus (Lombardi et al., 2004; Vroon et al., 2003). The varying effects observed in hyper-inflammatory states in RA and hypoxia/ischemia does not explain the therapeutic failure observed in animal models of inflammation treated with β-adrenergic antagonists (Guirguis et al., 2003; Kulmatycki et al., 2000). It is possible, however, that GPCR dimerization and /or oligomerization may lead to cross talk between various receptor and thus to down-regulation in effect.

Cross-talk between Receptors and Receptor Oligomerization

There is now a large and diverse body of evidence suggesting that GPCRs indeed function as dimers, or oligomers. Dimerization can occur among identical receptors, close family members, or between distinct families. They have been reported among β_2AR, the δ-opioid receptor, the chemokine receptor, the Ca^{2+} sensing receptor and the metabotropic glutamate receptor (Breitwieser., 2004). Receptors, which oligomerize are summarized in Table 1-2. Receptor dimerizations have been linked to GPCR sequestration, and appears to also affect ligand binding, receptor activation, desensitization and trafficking in a number of instances. It was mentioned above that second messenger PKC is able to independently phosphorylate GPCR via a heterologous signaling pathway. It appears that in human platelet activating factor monomer GPCR, PKC activation can enhance receptor multimerization, contributing to its desensitization/internalization (Perron et al., 2003). Effects of the oligomerization of the receptors on signaling appear widespread and varied. For example, during heart failure, expression of β_1AR is reduced, while β_2AR expression is increased. It may be that heterodimerization creates a negative crosstalk in which the expression of one receptor subtype is attenuated. As well, the complex interactions of multiple, branched signaling pathways will lead to positive and negative feedback, termed cross-talk. The effect of cytokines on the down-regulating effect on GPCR observed during varying inflammatory states appears, in part, to occur via the cross talk of the intracellular

Table 1-3: Functional consequences of GPCR Heterodimerization

Changes in Receptor Pharmacology	Co-desensitization or Internalization in Response to Partner Agonist	Altered Coupling to G Proteins and/or Signal Transduction Pathways; Synergy in Signaling
α_2AR-β_1AR	*α_2AR-β_1AR*	*AT$_1$R-B$_2$*
D1-A1a AR	*AT$_1$R-B$_2$*	*β_2AR-δOR, β_2AR-δ-κOR*
M2-m3	*β_2AR-δOR, β_2AR-δ-κOR*	*CCR2-CCR5*
δ-μOR	*TRH1-TRH2*	*δ-μOR*
δ-κOR	*β_1-β_2 AR*	*mGluR1a-A1AR*
	SST2a-SST3a	*mGluR5-A2a*
	SST2a-μOR	
	A2a-D2	

Adapted from Dzimiri N: Receptor Cross-talk. Implications for cardiovascular function, disease and therapy. *Eur J Biochem* 2002; 269: 4713-4730.

signaling proteins. In airway smooth muscle cells, cytokines IL-1β and TNFα coordinate with the epithelial growth factor to initiate 2 distinct signaling pathways to desensitize GPCR (Pascual et al., 2001). An increase in PKA due to PGE_2 is presumably thought to down-regulate the β_2AR. Cross talk is also seen in cardiovascular diseases, and may be one of the important causes of β_1AR decrease in function. In congestive heart failure rat model using MI, inotropic responses to β_1AR stimulant isoproterenol was reduced by 65 %, indicating GPCR desensitization, with no changes to receptor density. However, the expression of Gi versus Gs (in β_1AR) was greater, indicating the up-regulation in Gi from another GPCR signaling lead to β_1AR down-regulation in response (Kompa et al., 1999). Adenylate cyclase activity impairment in severe septic shock patients also appear to be due to heterologous desensitization mechanism of βAR and cytokine receptor cross-talk with βAR signaling mechanism, leading to significant decrease in cAMP levels and adenylate cyclase activity (Bernardin et al., 1998). In fact, evidence suggests increasing cytokine and cytokine receptors in advanced heart failure may increase cross talk between the cytokine signaling molecules and the GPCR in the heart (Deswal et al., 2001). Inhibiton of β-AR responsiveness may also be due to AGII stimulation of the AT_1R and subsequent PKC activation leading to NO production, and increased OP2 receptor activity (Dzimiri.,2002), leading to inhibition of β-AR stimulated AC activity via the Gi protein. Ca^{2+} regulation is also affected by the negative cross talk from various other receptors. The compartmentalization of the Gs stimulated cAMP signal from β_1AR, due to β_1-β_2AR heterodimerization, selectively affects plasma membrane effectors such as L-type Ca^{2+} channels (Xiao et al., 2001).

AT_1R and AT_2R exhibit negative cross talk as the signaling molecules downstream from the receptor have opposing effects on the body (Figure 1-4). However, AT_1R may very well have a positive cross talk with cytokine and growth factor receptors. Presumably, the tyrosine kinase pathway, through the JAK/STAT proteins, is similar between the AT_1R and growth factor receptors, leading to the possible shared response. Differential regulation however can be expected depending on the tissue and the stimulation conditions. Indeed, AGIIs pro-inflammatory activities may partially be through the induction of cytokine receptors. The therapeutic efficacy of AT_1R antagonists in inflammatory states may well be investigated as the signaling mechanisms of AT_1R vary from the βARs, as well as their expression and function in mediating the physiological system. Not much is known about the effect of inflammation on the therapeutic efficacy of AT_1R antagonists, especially in autoimmune conditions where much potential for variability exists in drug availability and efficacy at the receptor site.

Objectives of the Clinical Investigatio

The objective of our human study was to investigate the effect of inflammation on the pharmacokinetics and pharmacodynamics of CYP450 metabolized (activated) antihypertensive drug, losartan, and a non-CYP450 metabolized drug, valsartan. We chose rheumatoid arthritis as our model of inflammation as it is associated with an increase in cardiovascular events and because our previous studies on down-regulation of calcium channel receptors revealed abnormalities in this group of subjects. Three groups were chosen for the study: Active RA, Controlled RA and healthy subjects.

Hypotheses for the Clinical Investigation

1. Valsartan pharmacokinetics will not be altered with the presence of inflammation
2. Losartan pharmacokinetics will be altered with the presence of increased inflammatory state.
3. Decreased formation of the active metabolite EXP 3174 will be observed with patients treated with losartan.
4. The pharmacodynamic parameters for valsartan will not be altered in arthritic patients as compared to healthy subjects (ie. no down-regulation in AT_1R will be observed).
5. The pharmacodynamics for losartan will be altered due to a decrease in the active metabolite level, but not due to AT_1R down-regulation.

Depending on the results of the human study, we wished to investigate the mechanism of change in GPCR in inflammatory states. It was important, furthermore, to understand the effect of inflammatory state in an animal model with an underlying disease state. It was discussed previously how the incidence of cardiovascular events increase with the presence of inflammation, and that the cause of morbidity and mortality in RA patients in particular, is due to cardiovascular complications arising with the progression of arthritis and the increase in pro-inflammatory mediators (Solomon et al., 2003). RA patients are prone to essential hypertension, which eventually propagates into atherosclerosis and cardiac heart failure, despite the anti-hypertensive treatment. Although therapy failure due to increase in inflammatory mediator is suspected, studies so far have only investigated the possible mechanisms in a healthy animal model, without any underlying cardiac abnormality, in which acute and/or chronic inflammation is induced. Studies done so far also induce CV disease states in animals with underlying hypertension, but rarely is another type of inflammatory state induced to check drug effect in multiple disease state studies. Different animal models have different inflammatory response, which may not reflect what is occurring in the human body. It is therefore imperative to study disease-disease interaction, as well as drug-disease interaction as a more relevant animal model. The genetically hypertensive rat strains have become one of the mainstay models to study essential hypertension in humans.

Hypertensive Rat Model Strains

There are four major strains of rats with hypertension. These include the spontaneously hypertensive rat (SHR) developed by the Japanese workers, Okamoto and Aoki, in 1963 (Okamoto et al., 1963), a strain developed from Wistar rats in New Zealand by Smirk and Hall (1958), the Milan hypertensive strain developed from Wistar rats by Bianchi and co-workers (Bianchi et al., 1975), and the Dahl salt-resistant (R) and salt-sensitive (S) strains developed by Dahl and co-workers (Dahl et al., 1962).

In 1963 Okamoto and Aoki reported the development of a strain of Wistar rats selected for hypertension. The development of the SHR as a severely hypertensive strain of rat showed that hypertension took place over relatively few generations. By the F6 generation the level of blood pressure was reaching a plateau at about 180 mm Hg (Okamoto , 1969). Based on a genetic analysis by Tanase (1979) it appears that over 50 % of the increase in blood pressure in the SHR is associated with a single gene. With succeeding generations there has been a tendency for greater and earlier increase in blood pressure. The strain breeds true with 100 % of the offspring developing hypertension. It has become one of the more widely used strains inbred rats in the United States.

SHR have been claimed to be a good model for the study of essential hypertension in man since the condition is spontaneous, increases in severity with age, and is more severe in males. It is also often associated with complications of several organs including heart, brain, and kidneys, whose severity increase with age, similar to those found in human hypertension. In the brain there is often cerebral infarction, subarachnoid haemorrhage, and microscopic cerebral haemorrhages (The Laboratory Rat., 2000). Myocardial necrosis and microscopic fibrosis occur as well as nephrosclerosis and vascular lesions including fibrinoid necrosis and hyaline degeneration in renal, pancreatic, hepatic, and less commonly, coronary arteries. Early phase of hypertensive state appear to start from 6 weeks of age, however indications of end organ damage and cardiac hypertrophy do not appear until much later in life, at around 50 weeks or more.

The study of pathogenesis of hypertension oftern compares the hypertensive strains with their respective normotensive "controls", which are the Wistar-Kyoto rat (WKY) strain for the SHRs. However, there have been many differences reported in the literature when characteristics of the SHR have been compared with WKY. Genetic fingerprinting has determined that the SHR and WKY strains only share approximately 50 % of their DNA fingerprint bands (St. Lezin et al., 1992). The genetic difference would explain a number of significant differences, and illustrate one of the major problems in developing genetic models in providing a perfect control animal. The three major differences between the SHR and the normotensive controls may be significant with regard to the pathogenesis of the disease, and are as follow. The first of these relate to the level of sympathetic nerve activity. It appears that SHR has a greater sympathetic nerve activity than do age matched WKY (Judy et al., 1976; Nagatsu et al., 1974). The consequent hyperactivity observed with the SHR as compared to WKY may prove to be problematic in studies of drug response. Also, there is an increased wall to lumen ratio in SHR as compared to WKY and there is an increase in rate of protein synthesis in the vasculature even before the animals become severely hypertensive (Animal Models: Assessing the Scope of their Use in Biomedical Research., 1985). Finally, there is an apparent defect in the system for maintaining ionic homeostasis within various cell types (Meyer et al., 1983). Another interesting and significant finding is a marked immunodeficiency in SHR with nearly a 90 % loss of T-cells (Takeichi et al., 1981), although the contribution of T- cell deficiency in the hypertensive state has not been clearly stated.

Despite the enormous amount of work done with the SHR, the primary cause of its hypertension in the SHR is still unclear with different possibilities. In one case, defects in fluid and sodium reabsorption have been indicated to lead to hypertension. Renal transplant studies between SHR and WKY have shown that hypertension seems to follow the path of the kidney. For example, if an SHR kidney is placed in a WKY rat, the animal becomes hypertensive. If the reverse is done, the blood pressure of SHR will decrease (Uber et al., 1996; Kopf et al., 1993). Exaggerated salt and water retention due to reduced GFR, and a reduction in both total and fractional urinary sodium excretion is thought to be caused by abnormalities in several transport mechanisms, primarily in the proximal tubule (Beierwaltes et al., 1982). It does not fully explain its involvement in the vascular and sympathetic changes, which may contribute to hypertension as well. Although differences in strains is being slowly understood, clearly there is more work that needs to be done to fully adapt an acceptable model for studies in human hypertension.

Objectives for the Animal Study

The objective of the animal study was to establish a hypertensive-inflammation rat model, and compare it to the established inflammatory rat model in order to study the changes in pharmacokinetics and pharmacodynamics of cardiovascular drugs in inflammation. The Sprague Dawley Rat (SDR) inflammatory rat model, with the β-AR and K^+ channel blocker sotalol, was adapted to the spontaneously hypertensive rat (SHR) and the normotensive counterpart Wistar Kyoto rat (WKY).

Sotalol Pharmacokinetics and Pharmacodynamics

Sotalol ($C_{12}H_{20}N_2O_3S$) is a hydrophilic β-adrenergic blocker with a water/n-octanol partition coefficient (log p value) of 0.24 (Brugada et al., 1990). Unlike other β-adrenergic antagonists that are aryloxypropranolamines, sotalol enantiomers are methanesulfonamide-substituted phenethanolamines and thus are amphoteric. Therefore, the pKa values are 9.8 and 8.3 for the amine and sulfonamide.

Sotalol is a selective β-AR and K^+ channel blocker. It exerts its anti-hypertensive effects by decreasing the heart rate and affecting the cardiac conduction system, and prolongs the PR and the QT intervals in both human and the SDR model.

The oral bioavailability of sotalol racemate is 90-100% in humans (Anderson et al., 1999). The absorption rate is less than other β-adrenergic antagonists with peak concentrations occurring at 2-4 h (Singh et al., 1987). Sotalol does not undergo first-pass metabolism and is mainly eliminated unchanged via the renal route by glomerular filtration and tubular secretion, with a half life of 10-15 h (Singh et al., 1987). In humans protein binding is reported to be less than 2 % with albumin and AAG contributing to the binding (Belpaire et al., 1982). Diseases that alter renal excretion of sotalol will generally alter pharmacokinetics and pharmacodynamics as shown by the increased β-adrenergic and cardiac potassium channel blocking that may produce toxicity in patients with renal failure (Singh et al., 1987). The concentration effect relationships used to model the activities of sotalol are maximum effect (Emax) and sigmoid Emax models (Singh et al., 1987). Sotalol's pharmacokinetics allows development of optimal dosing for initiation of therapy relative to changes in creatinine clearance with further dose adjustment by monitoring the QT interval on the surface electrocardiogram.

Clinical Applications of Sotalol

Sotalol is administered as a racemate and is indicated for the treatment of hypertension, angina, and ventricular arrhythmias (Anderson et al., 1999). It has no intrinsic sympathomimetic or membrane stabilizing activities and elicits the antihypertensive/antiarrhythmic effect by non-selectively blocking β-adrenergic receptors and potassium channels in the myocardium (Anderson et al., 1999). Sotalol was first synthesized in 1960 and marketing began in 1974, therefore, racemic sotalol has been used as an antihypertensive and antianginal drug for more than 25 years. The antiarrhythmic activity of sotalol has resulted in renewed interest in the drug since trials such as the Cardiac Arrhythmia Suppression Trial (CAST) proved an increased risk of sudden death with class I antiarrhythmic agents. Sotalol inhibits the delayed rectifier and other cardiac potassium channels, and in 1992 the racemate was approved by the Food and Drug Administration for use in the treatment of life-threatening ventricular arrhythmias (Anderson et al., 1999). Interestingly, potassium channel blockers have been suggested to reduce mortality caused by left ventricular dysfunction after myocardial infarction (Waldo et al., 1996). Therefore, to determine whether d-sotalol, potassium channel antagonist, reduces mortality in patients with previous myocardial infarction and left ventricular dysfunction the Survival With Oral d-sotalol (SWORD) trial was conduced. This trial was stopped before completion due to a higher mortality in the sotalol treated group (Waldo et al., 1996). A possible reason for the increased mortality with d-sotalol, perhaps, is that β-adrenergic blockade is required to counteract the sympathetic activation that accompanies prolongation of the cardiac action potential (Adamson et al, 1998). Despite cancellation of the SWORD study there is renewed interest in d-sotalol as an agent to treat sustained ventricular tachycardia (Advani et al., 1995). Also, use of d-sotalol to decrease defibrillator energy requirements in patients with an automatic implanted cardioverter-defibrillator (AICD) is gaining interest due to the increased safety margin since lower energies for defibrillation are required (Dorian et al., 1996).

In inflammatory animal models, such as in the Sprague Dawley rat it was established that acute and chronic inflammation decreases the QT and PR prolongation observed with sotalol treatment (Kulmatycki et al., 2001).

We wanted to further establish *whether an underlying hypertensive state will have an effect on the pharmacodynamics of sotalol as compared to a normotensive state.* Based on the results and its comparability to sotalol effect in humans, the model can be further extrapolated and used to closely examine the AT_1R antagonist pharmacokinetics and pharmacodynamics. The SHR and the WKY rat models were picked because QT prolongation suggests the persistence of a higher risk of cardiovascular mortality that in patients that are borderline essential hypertensive as well (Kaftan et al., 2000). Similar ECG abnormality is observed in the SHR strain. A higher heart rate and inflammatory mediator level such as TNF, ILs and NO in the SHR strain has also been implicated in the formation of left ventricular hypertrophy, and increased mortality within 12 months. Induction of the acute inflammatory model may, in fact, worsen the electrocardiographic abnormality. Although the β-AR function and levels were not determined in SHR strain, the increase in pro-inflammatory mediators may in fact have an effect in decreasing sotalol antagonism on the β_1AR and K^+ channel blocking by negative cross-talk and hence down-regulation in sotalol effect may not be so evident in hypertensive state.

Hypotheses for the Animal Study

1. Sotalol potency on QT and PR interval prolongation will be diminished in the hypertensive SHR strain.
2. Acute inflammation will decrease sotalol potency on the PR and QT interval prolongation in the normotensive WKY and SDR strain, but inflammation will not alter sotalol effect in the SHR strain as compared to its controls.

Chapter 2: Experimental Materials and Methods

Assays for Pharmacokinetic Determination

High performance liquid chromatography (HPLC) assays were used for the measurement of drug plasma levels for valsartan and losartan in the human subjects. 1997).

HPLC Apparatus

An HPLC apparatus was used, consisting of a model 590 pump and a 712 Wisp autosampler. For valsartan, a 712 Wisp autosampler and a scanning fluorescence detector model 470 (Waters, Millipore, Mississauga, Canada) was used for sample detection. Losartan and EXP 3174 samples were detected using a UV detector model 470 (Waters, Millipore, Mississauga, Canada) with the wavelength was set at 254 nm. The apparatus also included a model 3390A recorder integrator (Hewlett-Packard, Palo Alto, CA, USA). A pre-packed ODS 10 cm × 4.6 mm I.D. C_{18} analytical column packed with 5-μm particles (Phenomenex, Torrance, CA, USA) attached to a NovaPak C_8 Guard-Pak HPLC Precolumn insert (Waters, Millipore, Mississauga, Canada) was used. The columns were operated at ambient temperature.

Assay for Valsartan

A novel HPLC assay was developed for the measurement of valsartan (Daneshtalab et al., 2002). Previous assays included gas chromatographic, mass spectroscopic and other HPLC methods requiring lengthy sample preparation involving solid-phase extraction, the use of commercially unavailable internal standards (Sioufi et al., 1994), or were non-specific as they were based on determination of radioactivity of ^{14}C compounds (Waldmeier et al., 1997). Our purpose was to develop a more simple and convenient assay employing commercially available internal standard for the quantitation of valsartan in human plasma using a liquid-liquid extraction procedure.

Sample preparation

Human samples for the valsartan assay were prepared as follow: A 100 µl volume of a 5 µg/ml losartan (internal standard) solution was added to 0.5 ml of patient sample and q.s'd to 1 ml with blank human plasma. Samples were then acidified to pH 2.5 with 125 µl of 1 M phosphoric acid (BDH, Edmonton, Canada). To the analytes were added 10 ml of methyl-tert-butyl ether (Fisher Scientific, Edmonton, Canada). The solutions were vortex-mixed for 3 min and centrifuged at 1800 g for 5 min. The organic solvent was transferred to clean tubes containing 200 µl of 0.05 M NaOH (pH > 10) (BDH, Edmonton, Canada), and vortex-mixed for 2 min. The aqueous layer was frozen by immersing the tubes in a dry ice-acetone bath. The organic layer was discarded and the aqueous layer thawed and neutralized with 75 µl of 0.2 M phosphoric acid (BDH, Edmonton, Canada). Aliquots of 125 µl were injected into the HPLC.

Quantification

Standard curves were prepared using known concentrations of valsartan powder, kindly provided by Novartis Pharma (Basel, Switzerland). Concentrations of 10, 70, 100, 500, 1000 and 2000 ng/ml of valsartan were prepared using varying volumes of the working stock solutions of the drug. A 100 µl aliquot of the 5µg/ml internal standard and 1 ml of blank human plasma were also added and extracted as described above. The area under the HPLC response peak was recorded for the analytes and the drug/internal standard peak ratios were plotted versus concentrations. Linear regression was used to estimate the best fit.

Extraction efficiency, accuracy, and precision

To calculate the extraction efficiency, solutions for various valsartan concentrations were made using the above-mentioned valsartan stock concentrations, were extracted as described above, and aliquots of 125 µl were injected into the HPLC. The peak areas of samples were compared with that obtained after direct injection of unextracted valsartan solutions. To determine the variability of the intra-day and inter-day extraction, aliquots of blank plasma were spiked with valsartan and the internal standard to make final concentrations of 10, 100, 500, 1000, 1500, and 2000 ng/ml. The samples were then extracted according to the developed method. The accuracy and precision for the intra-day and inter-day samples were determined as the % recovery (found concentration/given concentration x 100 %) and the % coefficient of variation (C.V), respectively.

An excellent linearity was observed (r^2 >99) between reponse and concentration within the examined range of 10-2000 ng/ml. Equations describing typical standard curves of the low (10-500 ng/ml) and high ranges (500-2000 ng/ml) were y=0.0199x-0.0071 and y=0.0209-0.3172 respectively. The limit for quantitation for valsartan was 10 ng/ml (CV <11%), with percent accuracy of 96 ± 4 % based on 1-ml plasma sample, which was comparable to those previously reported. However, solutions containing as low as 2.5 ng/ml exhibited a signal-to-noise ratio of 5. The average extraction efficiency for concentrations of 10, 100 and 1000 ng/ml valsartan was 69 ± 4.3 %. In addition, the observed accuracy and precision (Table 1) were well above the acceptable limit and comparable with other reported methods (Sioufi et al., 1994).

The mobile phase consisted of 70% pH 2.8 phosphate buffer and 30% acetonitrile (Fisher Scientific, Edmonton, Canada), pumped at a flow-rate of 1.3 ml/min. Standard curve was linear over a 10- 2000 ng/ml range. Losartan was used as the internal standard (Merck., Rahway, NJ, USA). The internal standard and valsartan sample were eluted out at ~10 min and ~26 min respectively (Figure 2-1).

Table 2-1:

Accuracy (% error) and precision (coefficient of variation: % C.V.) of the valsartan spiked solutions

Added (ng/ml)	**Inter-day variability (n=4)**		C.V. (%)	**Intra-day variability (n=6)**		C.V. (%)
	Concentration (ng/ml)			*Concentration (ng/ml)*		
	Observed	*% Error*		*Observed*	*% Error*	
10	9.9	99.3 ± 10.8	10.8	10.0	99.8 ± 8.1	8.9
100	96.5	96.5 ± 6.8	7.1	95.3	95.3 ± 7.3	8.4
500	465.8	93.2 ± 8.7	9.4	456.1	91.2 ± 7.3	8.8
1000	917.8	91.8 ± 2.4	2.6	883.1	88.3 ± 5.2	6.6
1500	1470.1	98.0 ± 2.2	2.2	1472.7	98.2 ± 1.9	2.1
2000	2012.9	100.6 ± 0.7	0.7	2011.3	100.6 ± 0.9	0.8

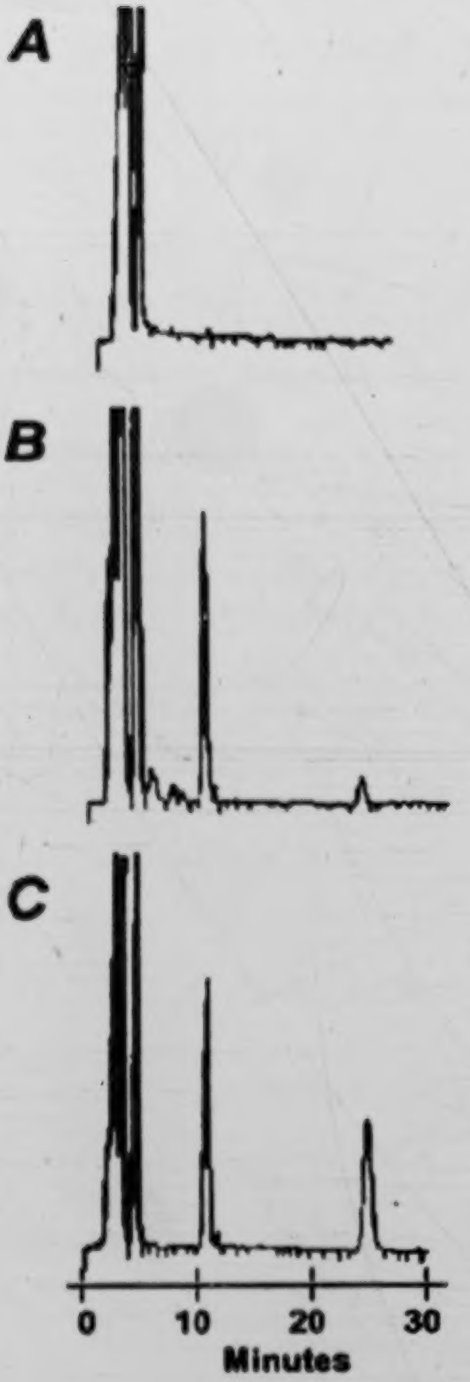


Figure 2-1: Examples of chromatograms obtained from an extract of 1 ml blank human plasma (A), sample plasma spiked with 10 ng/ml of valsartan (B), and a sample from a patient 5 h post-dose (C). Losartan and valsartan peaks are depicted at ~ 11 and ~26 min after injection into the HPLC system.

Assay for Losartan

The previously published HPLC assay was slightly modified for the measurement of losartan and EXP 3174 (Ritter et al., 1997). Briefly, a 100 μL aliquot of the 1 μg/mL of the internal standard was added to 0.5 mL of patient sample and quesced to 1 mL with blank human plasma. Samples were then acidified to pH 2.5 with 125 μL of 1 *M* phosphoric acid. To the analytes were added 10 mL of methyl-*tert*-butyl ether. Solutions were vortex-mixed for 3 min and centrifuged at 1800 *g* for 5 min. The organic solvent was transferred into clean tubes containing 200 μL of 0.05 *M* NaOH (pH > 10). After vortex mixing and the centrifugation process, the aqueous layer was frozen by immersing the tubes in a dry ice-acetone bath. The organic layer was discarded, the aqueous layer thawed and neutralized with 75 μL of 0.2 M phosphoric acid. Hexane (5 mL) (Fisher Scientific, Edmonton, Canada) was then added followed by vortex mixing for 1 min to wash the aqueous fraction. The hexane was discarded by further centrifugation and freezing process in acetone/water. To improve solubility, 75 μL isopropranolol was added to the remaining aqueous sample. Aliquots of 125 μL were injected into the HPLC.

Losartan mobile phase consisted of 0.015 M H_3PO_4, acetonitrile and tri-ethyl amine (TEA) (Fisher Scientific, Edmonton, Canada) in a ratio of 71:29: 0.078 and pumped at a flow-rate of 1.3 mL/min. Standard curve was linear over a 10- 2000 ng/ml range with a CV <10 %. The elution times for losartan, internal standard and EXP 3174 were 13 min, 17 min and 27 min respectively. Samples were compared with standard curves prepared using known concentrations of losartan, EXP 3174 and Internal standard, kindly provided by Merck Research Laboratories (Rahway, NJ, USA). The minimum quantifiable concentration was 2.5 ng/ml with a CV <15%.

Inflammatory Determination Assays

Nitric Oxide determination

Total nitrite (NO_2^-) a stable breakdown product of both NO and $ONOO^-$, is an indirect measure of reactive nitrogen species levels in plasma. As the ultimate metabolic fate of NO *in vivo* is nitrite (NO_3^-) it must be reduced to nitrite by nitrate reductase for measurement using a method reported by Archer and Grisham (Archer et al.,1995; Grisham et al., 1995). Briefly, 100 µL of plasma was incubated for 30 min at 37 °C in the presence of 10 U/ml *Asperigillus* nitrate reductase, 1 M HEPES buffer, 0.1 mM flavine adenine dinucleotide (FAD), 1 mM nicotinamide adenine dinucleotide phosphate (NADPH) in a total of 500 µL to reduce all the NO_3^- to NO_2^-. The reaction was then quenched with 1500 U/mL lactate dehydrogenase (LDH) and 50 µL of 100 mM pyruvic acid for 10 min. The reaction oxidized any unreacted NADPH remaining and prevents interference with Griess reaction. The above-mentioned chemicals for NO determination were purchased from Sigma Chemical Co (St.Louis, MO, USA). One mL of premixed Griess reagent (1:1 of 0.2 % [w/v] naphthyleneethylenediamine and 2 % [w/v] sulfanilamide in 5% [v/v] phosphoric acid) was then added, and after 10 min incubation, the absorbance was measured at 540 nm using a Powerwave x 340 plate reader (Bio-Tek Instruments, Fisher Scientific). Calibration was performed using standard solutions of $NaNO_2$ and $NaNO_3$. A comparison of $NaNO_2$ and $NaNO_3$ calibration curves was used to test the dehydrogenase efficiency. The assay was linear from 5-200 µM with a CV <10%.

C - Reactive Protein determination

CRP is one of the acute-phase proteins whose plasma levels rise during general, nonspecific response to infectious and noninfectious inflammatory process. Not only can

CRP levels be indicative of an acute and chronic inflammatory processes, rising from 0.5 mg/L to 20-500 mg/L within 4-8 h after an acute event, it may aid in the assessment of the risk of cardiovascular and peripheral vascular disease.

For the human study, the Dade-Behring (Deefield, IL) assay kit, using a nephelometric measuring method, was used at the University of Alberta Hospitals to determine CRP levels. This high-sensitivity CRP assay consists of a suspension of polystyrene particles coated with mouse antibodies to human CRP. The BN^{TM} II nephelometer then measures the concentration of suspended particles optimal for agglutination. The CRP content can be determined by the addition of reagents, which allow variable scatter of light depending on the concentration. The values would then be compared with prepared standard concentrations.

Segmented Neutrophil count.

As a measure of inflammatory response to $IFN_{\alpha 2a}$, white blood cell counts were performed using fresh blood withdrawn from the rat-tail. The amount of segmented neutrophils was counted since activation of the inflammaotory response is thought to accelerate the maturation process (Dahlgren and Karlsson., 1999). In addition, administration of $IFN_{\alpha 2a}$, is reported to enhance neutrophil respiratory burst, a step in which oxidative metabolism of neutrophils increase before phagocytosis, which occurs with bacterial and viral infection (Little et al, 1994). As well, oxidative burst responsiveness by neutrophils correlates with severity of inflammation (Hansen et al., 1999). Fresh blood was placed on a microscope slide and spread evenly throughout the whole slide to form a very thin layer of blood. The smear was then air-dried at room temperature for 15 min, and covered with differentiating Jenner stain (1:1 Methylene blue and Eosin Y) for 30 to 60 sec.

The slides were then placed in Sorenson's phosphate buffer (Sorensen SPL, 1909) (0.067M KH_2PO_4 and 0.067 M $Na_2HPO_42H_2O$) for 30 min and washed with distilled water. A total of 100 white cells were counted to determine the percentage of segmented neutrophils.

Human Clinical Study Protocol

Ethics and Consent

The study was performed in accordance with the Declaration of Helsinki. The protocol was approved by the University of Alberta Hospital Research and Ethics Committee. All participants provided written informed consent. Subjects were recruited from rheumatology clinics in the city of Edmonton, Alberta.

Inclusion Criteria

Subjects meeting all of the following criteria were included in the study:

- Males or females of non-childbearing potential (birth control pill [must have been on for 3 months], IUD, post-menopausal or surgical sterilization)
- Clinical laboratory values outside the laboratory's stated normal range unless the Clinical Investigator decides the abnormalities are not clinically significant and records this fact on the CRF.
- Healthy (except for rheumatoid arthritis) according to the laboratory results and physical examination. Patients with known hypertension were not included.
- Non-smoker for at least 3 months

Exclusion Criteria

Subjects meeting one of the following criteria were excluded:

- History of hypersensitivity to any angiotensin II antagonists
- History of angioedema due to ACE inhibitors
- Significant history of presence of gastrointestinal liver or kidney disease, or any other conditions known to interfere with the absorption, distribution, metabolism or excretion of drugs.
- Significant history of asthma, chronic bronchitis, other bronchospastic conditions
- Significant history of inflammatory disease of the gastrointestinal tract such as peptic ulcer disease, gastritis, esophagitis, regional enteritis or ulcerative colitis.
- Significant history of allergies
- Significant history of presence of cardiovascular or unexplained haematological disease.
- Presence of diabetes mellitus or any other condition, which would preclude fasting
- Maintenance therapy with any drug (except those prescribed for rheumatoid arthritis, or antihypertensives), or history of drug dependence, alcohol abuse, or serious psychological disease. Antihypertensive drug use is not exclusion provided no more than two antihypertensive drugs are being used and the original hypertension severity was either mild or moderate. Antihypertensives must be able to be safely withdrawn for one week prior to, and during, the sampling periods.
- Use of an angiotensin II antagonist within 30 days prior to the start of this trial.

- Any clinically significant illness other than rheumatoid arthritis in the previous 30 days before day 1 of the study
- Use of enzyme-modifying drugs in the previous 30 days before day 1 of this study (All barbiturates, systemic corticosteroids, di and methyl-phenylhydantoin, etc)
- Blood donation in the previous 56 days or multiple blood sampling in the previous 30 days before day 1 of this study
- History of fainting upon blood sampling
- Participation in another clinical trial within 30 days of the start of this trail

Subject Selection and Protocol Summery

A total of 38 subjects, in the age range of 22-74 year, were entered into the study. The subjects were divided into three groups: 14 patients with active rheumatoid arthritis, 12 patients with controlled rheumatoid arthritis, and 12 healthy subjects as controls. Patients were selected independent of blood pressure. However, known hypertensives were excluded and study subjects were not on any cardiovascular drugs.

The subjects all underwent routine laboratory tests one week prior to the commencement of the study. The screening studies consisted of hematology, routine biochemistry, ECG, urinalysis, and C-reactive protein levels.

Patients were matched between groups based on the arthritis therapy used. They were diagnosed according to the American Rheumatism Association 1987 revised criteria (Schumacher et al., 1993). Arthritic index was calculated using number of joints involved and the level of severity. Subjects were dosed with 160 mg capsules of valsartan or 100 mg tablets of losartan with a one-week washout period in between. The AT_1R antagonists were purchased from the University of Alberta Hospital Pharmacy.

On the study day, subjects arrived before 0700 to the clinical investigation unit after at least an 8 h fast. An intravenous line was inserted for blood sampling and the patient allowed to rest recumbent for 30 min prior to measuring baseline physiological variables. Physiological variables included: mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP), pulse, cardiac ejection time (card ej) calculated stroke volume index (SV, SVI), calculated cardiac index (CO, CI), peripheral vascular resistance (PVR), large artery compliance (LE), small artery compliance (SE) and total vascular impedance (TVI). All measurements were carried out using an HDI/Pulsewave™ Cardiovascular Profiling Instrument CR-2000 (Hypertension Diagnostics Inc., Minneapolis, MN). The percent change from baseline, the maximum percent effect and the area under the percent effect curve (AUEC) were calculated for the cardiovascular parameters.

No arthritic medications were taken within 24 h of the study, and all subjects fasted on the evening prior to the study. If necessary, acetaminophen was given for pain control. Acetaminophen does not interfere with pharmacokinetic analysis of valsartan or losartan. For ethical reasons, subjects could not be without anti-rheumatoid arthritic medication for longer than 48 hours. Most actively treated patients were receiving methotrexate (12/14 in the acute group, 6/12 in the chronic group) with some patients receiving other or a combination of agents: gold (2 patients), chloroquine (2 patients) and salazopyrin (2 patients). All patients took a variety of non-steroidal anti-inflammatory drugs as necessary in addition to the preceding remitive agents.

After baseline measurements were carried out and a blood samples taken for the measurement of NO levels, the subjects were given the respective drugs along with 250 ml of distilled water. Valsartan (Diovan, Novartis Switzerland) 160 mg capsules were purchased from the University of Alberta. For arthritic subjects (active and remission) lot number 009100 was used. For normal subjects, lot number COB03081 was used. Losartan (Cozaar, Merc Frosst) 100 mg tablets were purchased from the University of Alberta. For arthritic subjects (active and controlled arthritis group) lot number C012160 was used. For healthy subjects, lot number F010760 was used. Drug administration was double-blinded and the order randomized. After drug administration, subjects were required to remain upright for a minimum of two hours. At two hours subjects received 240 ml of orange juice and were then free to drink at will. At four hours and nine hours, standard meals were provided. No other food was consumed other than that provided. After the final sampling, subjects left the clinical investigation unit to return in one week for the second period.

Blood samples (approximately 10 mL) were taken and pharmacodynamic measurements were carried out at 0, 0.5, 1, 1.5, 2, 3, 4, 5, 8, and 12 h. Blood was centrifuged immediately after collection and the plasma immediately frozen at –70°C. Pharmacokinetic indices were calculated using the model independent approach. Oral clearance (CL/F), oral volume of distribution (Vd/F), area under the plasma concentration vs. time curve ($AUC_{0-\infty}$), terminal elimination rate constant (β), terminal half life ($t_{1/2}$), maximum plasma concentration (C_{max}) and the time of its attainment (T_{max}) were estimated. Plasma nitrite levels were measured in the time zero blood samples. As well, a concentration versus percent effect change from baseline assessment was performed on the average values for each group.

Power Of the Study

The power of the study was calculated to detect a significant difference in the pharmacokinetic and pharmacodynamic parameters. At the power of 0.8, the study will be able to detact a significant difference in Cmax and AUC $_{0-t}$, a 6.3 mmHG difference in blood pressure, and a 20 % difference in other cardiac parameters, assuming usual measurement standard deviations and an α of 0.05. The calculations were determined using the following formula:

$$Z_\beta = \sqrt{{}^{N \times \delta_2}\!\big/_{2 \times \sigma_2}} - Z\alpha$$

where N is sample size, δ is the maximum difference (20 %) accepted in the mean values for the variables studied, σ^2 is the mean squares residual, and Zα is the value corresponding to a two-tailed α of 0.05 (1.96). Zβ and the corresponding one-tailed β value are calculated, therefore giving the power (1-β).

Statistical Analysis for the human study

The SAS program, as well as the Microsoft Exel stastical program, was used for calculations. Statistical significance of the observed differences were tested using the one way ANOVA followed by the post-hoc Duncan's New Multiple Range test at α = 0.05 for two and more means. For AUEC in which each data point was comprised of multi-measurement, the standard error of mean was calculated. All other indices are expressed as mean ± standard deviation. The unpaired and paired Student's t-test was used to compare whether the baseline and post-treatment blood pressure measurements were significantly different from one another.

To test the significance of correlation between degree of inflammation and parameters, a least-squares linear regression was performed using a Pearson's correlation coefficient to determine goodness of fit.

Rat Study Protocol

Animals and dosing

The investigation was performed in adherence to the principles of the Animal Ethics Committee of the University of Alberta. Three strains of adult male rats were all purchased from the Health Sciences Laboratory. The strains consisted of Sprague-Dawley (n= 8, 311.7 ± 4.2 g), Wistar Kyoto (n = 11, 284.1 ± 27.1 g) and Spontaneously Hypertensive rats from Charles River (n = 12, 274.8 ± 11.6 g). They were all housed in standard rodent cages, kept on a 12 h light/dark cycle, and fed a standard diet of Purina rat chow.

Induction of inflammation

Each strain was divided into two groups. Acute inflammation was induced to one group by 2 subcutaneous injections of 5.0×10^4 units of $IFN_{\alpha 2a}$ (Roferon A, Roche Pharmaceuticals, Mississauga ON, Canada) using a 21 gage needle at 12 and 3 h prior to sotalol administration. The $IFN_{\alpha 2a}$ used is manufactured by recombinant DNA technology using genetically engineered *Escherichia coli* contaning DNA coding for human protein. The second, non-inflamed group received equi-volume of saline instead of $IFN_{\alpha 2a}$. Inoculation time for each strain and group were at the same time as to prevent the diurnal rhythms of pro-inflammatory cytokines (Petrovsky et al., 1998; Loubaris et al., 1983).

To determine the severity and the presence of inflammation, three sets of blood samples were taken using tail-vein sampling from the veins located on the lateral surface (either side) of the tail. Tail was first wiped with gauze soaked with warm antiseptic soap and water. To allow the vein to dilate, sampling was performed under a heat lamp. The tail was straightened between the thumb and index finger and the 21G needle was inserted in a 30-45 degree angle from the distal most part of the tail. Since the vein is very shallow, deep insertions were not necessary. The first sampling period was prior to the commencement of $IFN_{\alpha 2a}$ and/or saline injection to determine baseline levels of inflammatory mediators. The second sample was taken just prior to the second injection of $IFN_{\alpha 2a}$ / saline. The last blood sample was taken at the end of the experiment. In total, approximately 0.5 mL blood was taken to determine the severity of inflammation. Each sampling was done using 1 mL syringes coated with 10 U/ml of heparin. These blood samples, in heparinized eppindorf tubes, were centrifuged for 3 min at 35,000 rpm and the plasma was separated and frozen at -70 °C until use. A drop of the blood sample was used for differential staining and segmented neutrophil count. The percent segmented neutrophils was determined since activation of the inflammatory response is thought to correlate with severity of inflammation (Hansen et al., 1999).

Drug administration

Racemic sotalol [(L)GD8741, exp April 2004] 80 mg tablets were a gift from Central Care Pharmacy (Edmonton, Alberta, Canada). They were administered by grinding the tablets using a mortar and pestle and dissolving in normal saline. Appropriate volume was given to the rats/weight, to a dose of 80 mg/kg using oral gavage.

Eectrocardiographic Measurements and Analysis

At 12 h prior to induction of inflammation, rats were anaesthetized using ethyl ether. Three braided stainless steel Teflon coated electrodes (Cooner Wire Co, Chaterworth, CA) were placed subcutaneously in the left and right axilla and over the xyphoid process (Figure 2-2). The rats were allowed to recover from the anesthetic and baseline ECG was measured for 5 h. The lead II 5 electrocardiogram was recorded using a Hewett-Packard Digital Holter with full disclosure (Hewlett-Packard, Avondale, PA). The ECG amplifier was a Honeywell for Medicine ECG amplifier (Honeywell Electronics for Medicine, Edmonton, Canada) and the data recorded using Acknowledge software (World Precision Instruments, Miami FL) on a personal computer. The mean of five cycles was taken for the measurement of PR and QT intervals and heart rate (HR). QTc was calculated by QT/(SQRT(60/RR)) using Bazett's formula. The PR interval was measured as the distance from the base of the P wave to the base of the R wave. The PR interval represents the time required for an impulse to conduct through the tissues located above the ventricles. i.e., atria, AV node and HIS bundle. The RR interval is the distance from the crest of one R wave to another, and this represents heart rate. In the rat, the ST segment of the ECG is often a plateau and not clearly defined. The quantification of the QT interval was therefore the distance from the Q dip to the bottom of the ST segment. The QT interval represents the conduction through Purkinjie fibers and ventricular muscle represents ventricular depolarization and repolarization and is used to assess

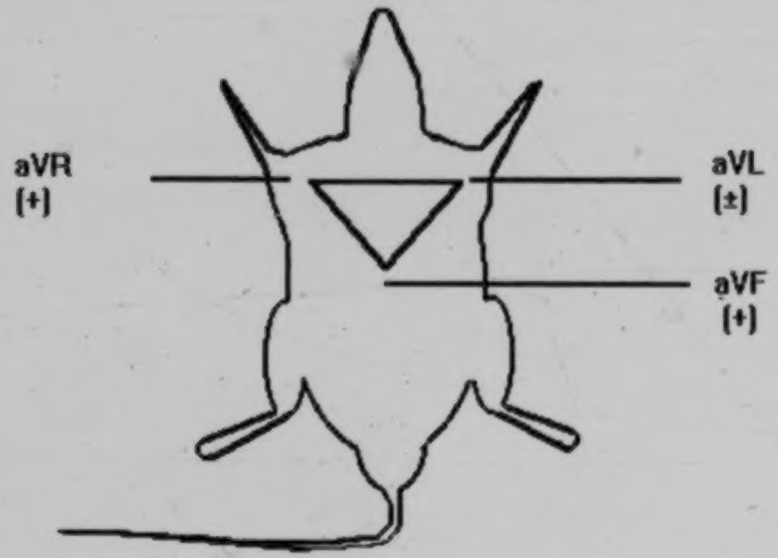


Figure 2-2: Electrode placement for the rat ECG.: a, augmented; V, voltage; R, right; L, left; F, foot

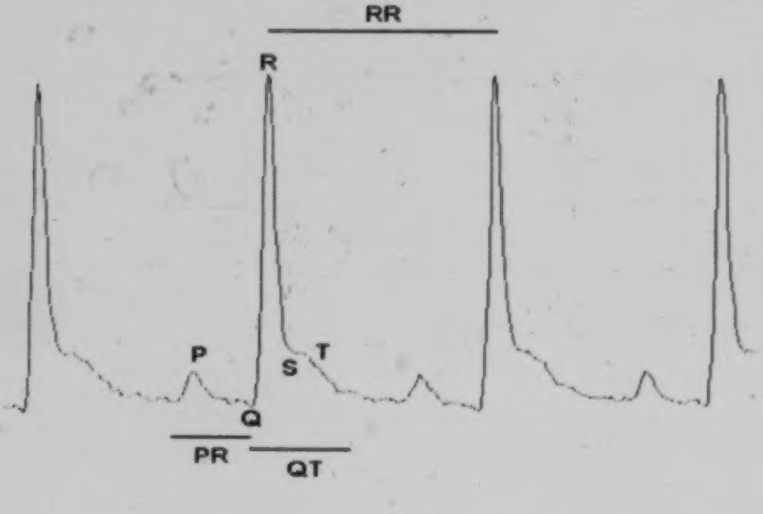


Figure 2-3: Typical rat ECG : illustrating P-Wave, QRS complex and T-Wave.

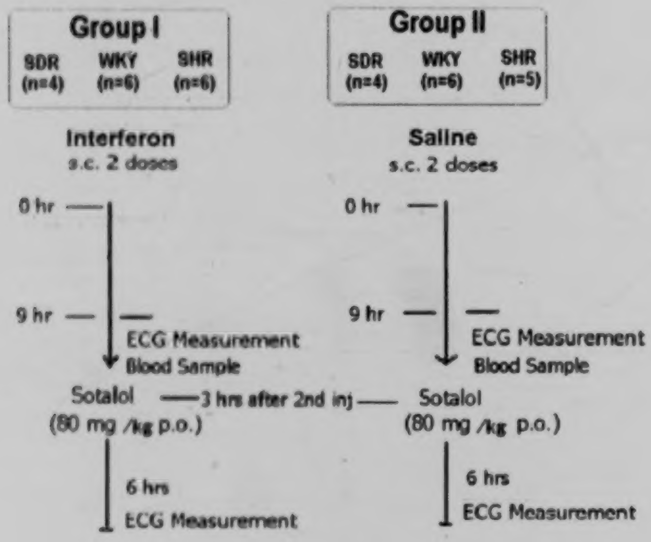


Figure 2-4: Experimental Protocol Summery for the rat experiment.

cardiac potassium channel blocking activity. Figure 2-3 depicts the typical rat ECG and the ECG parameters measured are indicated as well. The percent change from baseline, the area under the percent change from baseline versus time and the maximum percent change from baseline were calculated. Comparisons were made between the control and inflamed group within a strain, or between different strains within one group. Figure 2-4 depicts the protocol summary for the rat experiments.

Western blot techniques

Homogenization of the rat hearts

The cardiac cell preparations were obtained based on conventional methods. All the procedures were carried out in the cold room, and all the buffers, cocktail solutions and tissues were kept on ice during the course of experiment.

One week after the experiment, rat hearts from each strain was isolated after cardiac puncture and heart exsanguinations and immediately placed in a bottle kept in dry ice. Each heart sample was then cut into small pieces placed in freshly prepared ice-cold Tris-Cocktail buffer (10 mL/g wet tissue). The buffer contained 0.2 M Tris-HCl buffer solution and protease inhibitor cocktail (0.5 mL/10 mL buffer) (Sigma, Saint Louis, Missouri, USA). Homogenization of the heart was performed using an Ultra Turrex T25 homogenizer at speed of 20'000 rpm for 30 sec with the tube immersed in ice bath. The crude homogenate was placed in clean plastic tubes and centrifuged at 5000 rpm for 10 min to sediment the nuclei and cytoskeletal elements. The supernatant was separated and transferred into clean tubes and frozen at -70°C. An aliquot was used to determine the protein concentration by the method proposed by Bradford, using bovine serum albumin at 10 mg/10 mL for standard preparation (Bradford et al . 1976). Samples were diluted to 1/500th of their concentration and performed in triplicate. Eight hundred μL of each standard stock or sample was then taken and 200 μL of Bio Rad protein assay dye was used for the colorometric detection of the protein was performed at 540 nm wavelength using a Powerwave x 340 plate reader (Bio-Tek Instruments, Fisher Scientific).

β_1AR Protein Density Level Determination

To study the expression of β_1AR, volumes of heart homogenate supernatants corresponding to 50 or 100 µg of total protein were mixed with equal volumes of sample buffer (50 µL of β-mercaptoethanol and 950 µL of Laemmeli's sample buffer) (BioRad, Hercules). These solutions, along with 15 µL broad range pre-stained marker proteins (PSM) /gel (New England Biolabs) were immersed in boiling water for 3-5 min and loaded into 10 % SDS-PAGE gel. Using running buffer (Tris, 25 mM/glycine, 192 mM/0.1 % SDS buffer) the gel was run at 75 volts for 20 min and at 200 volts for 30 min at room temperature. Following electrophoresis, the proteins on the gel were transferred to a nitrocellulose membrane using transfer buffer (Towbin buffer: Tris 5 mM/glycine, 192 mM) at 100 volts for 1 h. The membrane was washed 4 times at 10 min intervals in TPBS (1 % Tween in phosphate buffered saline). Non-specific binding sites were blocked by immersing the membrane in 5 % skimmed milk powder suspended in TPBS overnight at 4°C. The membrane was incubated with the primary rabbit anti-rat β_1AR polyclonal antibody (1/500 dilution in 5 % milk suspension in TPBS buffer) for 1 hr. After thoroughly washing the membrane in four times in ten min intervals with TPBS wash buffer, it was further incubated with the conjugated Horse Radish Peroxidase –goat anti-rabbit IgG [H + L] secendary antibody (1/10,000 dilution in 5 % milk suspension in TPBS buffer) for 30 min at room temperature. In order to confirm the accuracy of sample loading into the wells, the density of β-actin is used as an internal standard. After another set of washing with TPBS (four times ten min intervals), the membrane was incubated with goat anti-rat β-actin polyclonal antibody (Actin [I-19], 1/2000 dilution in 5 % milk suspension in TPBS buffer) for 30 min followed by washing with four changes of TPBS buffer in 10 min intervals. The secendary antibody (1/50,000 dilution of HRPO-conjugated rabbit anti goat IgG [H + L] in 5 % skim milk suspension in TPBS buffer) was then added to the membrane and incubated for 30 min. The β_1-actin bands would be present at 42 KDa. Antibody binding at 64 KDa is indicative of β_1-AR protein. Enhanced Chemi-Luminescence (Amersham Biosciences) system kit was used for the detection of the protein bands. The principle of ECL detection system uses measurement of the light emission resulting from the dissipation of energy from a substance in an excited state affected by a chemical reaction. The reaction measures the HRP/Hydrogen Peroxide catalyzed oxidation of luminol in alkaline conditions. Immediately following oxidation, the luminal is in an excited state which then decays to ground state via a light-emitting pathway. Oxidation of luminal by the HRP in the presence of chemical enhancers such as phenols will increase the light output approximately 1000 fold, which peaks after 5-20 min and decays slowly. The light radiation is then detected on a sensitive radiographic film. Equal volumes of reagent 1 and 2 were mixed. The final volume was made to be 0.125 ml/cm^2 and the blot was incubated for one min in the solution. Excess amount of ECL was tapped off and the blot was placed on a clean piece of saran wrap and wrapped with care as to rid of all bubbles. The blot was then fixed unto developing cassette and developed in the dark room using Kodak film at various exposure times.

Statistical Analysis for the Rat Study

The SAS program and the Microsoft Excel statistical program were used for statistical analysis. For baseline differences in ECG parameters between strains, in which multi-measurements were made for each rat in each strain, the standard error of mean was calculated. All other indices are expressed as mean ± standard deviation. Inter-strain and intra-strain significant differences in inflammatory mediator levels and the pharmacodynamic parameters measured were tested using the one way ANOVA followed by the post-hoc Duncan's New Multiple Range test at $\alpha = 0.05$ for two and more means. The Student's t-test for unequal variances was used for comparison between $IFN_{\alpha 2a}$ and saline treatments within the strains.

Chapter 3: Human Study Results and Discussion

Results:

Subject Characteristics

Subject characteristics are shown in Table 3-1. There were no significant differences between groups in age, height, and weight. Baseline blood pressure indices did not differ significantly among the subjects. For the arthritic groups, the arthritic medications taken by the subjects are not known to interfere with the pharmacokinetics or the pharmacodynamics of losartan or valsartan.

Inflammatory Mediator Levels

The activity of the rheumatoid arthritis was based on arthritis index, CRP and nitrite levels (Figure 3-1). TNFα levels were also measured in all subjects, however results were not indicative of true levels. Sample degradation due to improper storage conditions, for a period of more than 8 months, may account for the distortion in the results (Biosource International). Therefore, TNFα results were not included. Arthritic index was significantly different between the three groups, as significantly higher numbers of joint inflammation were determined in patients with active inflammation (Table 3-2, Figure 3-1). Higher pro-inflammatory levels were also observed, as plasma NO_2^- and CRP levels were significantly elevated in patients with active rheumatoid arthritis as compared to those with controlled arthritis and healthy subjects (Table 3-2, Figure 3-1).

In addition, plasma CRP ($r = 0.46$, $p < 0.05$, $n = 38$) and NO_2^- ($r = 0.53$, $p < 0.05$, $n = 38$) significantly correlated with disease severity expressed as arthritic index (AI) (Figure 3-2). Also, there was a positive correlation when the increase in CRP levels was compared with NO_2^- levels ($r = 0.37$, $p < 0.05$, $n = 38$). In all correlations, the slope of the regression line was relatively flat.

Table3-1: Characteristics of subjects. Mean ± SD presented.

	Control (n=12)	**Active arthritis** (n=14)	**Controlled Arthritis** (n=12)
Age (Years)	56 ± 12	51 ± 13	51 ± 17
Male/female ratio	3/9	10/4	7/5
Height (cm)	167.5 ± 13.5	30.3 ± 8.7	27.6 ± 3.3
Weight (kg)	86.1 ± 20.5	88.6 ± 21.4	81.4 ± 12.7
Body Mass Index	30.6 ± 5.6	30.3 ± 8.7	27.6 ± 3.3
Baseline Blood Pressure (mmHg)			
SBP	131.3±13.3	129.3±12.3	120.8±14.4
DBP	72.6±9.8	74.2±6.8	73.0±13.2
MAP	94.3±10.0	95.1±9.9	91.4±15.4
Other Medications	none	Methotrexate (12/14); Hydroxychloquine (1/14); Gold (2/14)	Methotrexate (6/12); Hydroxychroloquine (2/12); Gold (2/12); Salazopyrin (2/12);

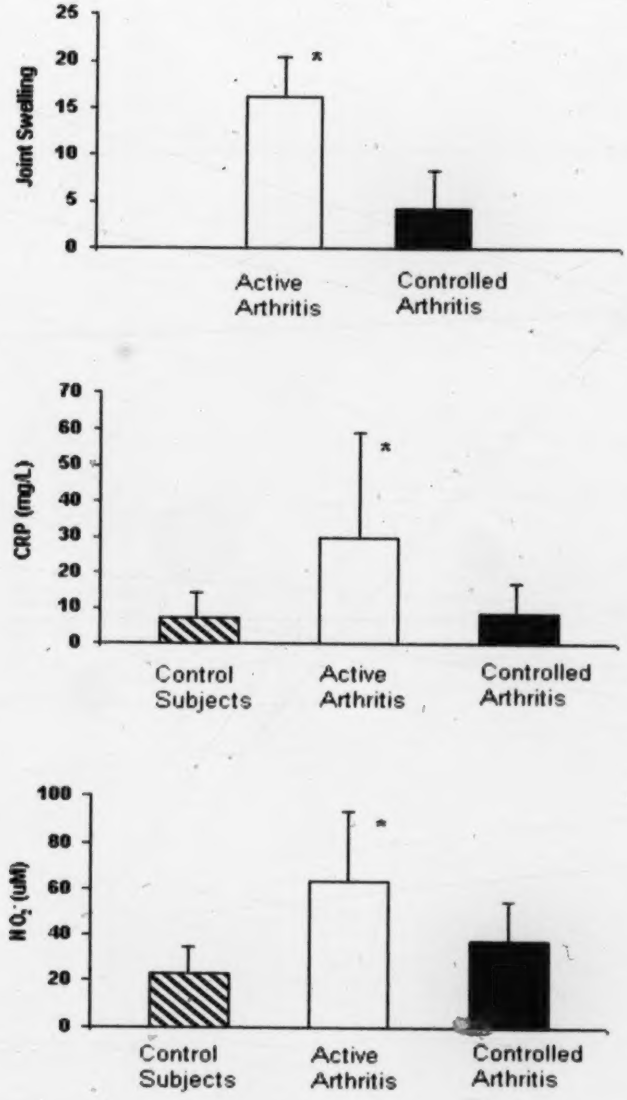


Figure 3-1: Mean ± SD of markers of inflammation, Arthritic Index, C-reactive protein, (CRP) and nitrite (NO_2^-).

* Significantly different from other groups (one way ANOVA followed by the Duncan's New Multiple Range test at $\alpha = 0.05$). Control (n=12), Active Arthritis (n=14), Controlled Arthritis (n=12).

Table 3-2: Mean ± SD of inflammatory mediator characteristic of subjects.

	Control (n=12)	**Active RA (n=14)**	**Controlled RA (n=12)**
CRP (mg/L)	**7.2 ± 6.8**	**30.1 ± 29.1***	**8.7 ± 8.0**
NO_2^- (μM)	**22.8 ± 12.2**	**63.5 ± 29.9 ***	**37.7 ± 17.2**
Arthritic Index	**0**	**16 ± 4***	**5 ± 4**

* Significantly different from other groups (one way ANOVA followed by the Duncan's New Multiple Range test at α = 0.05).

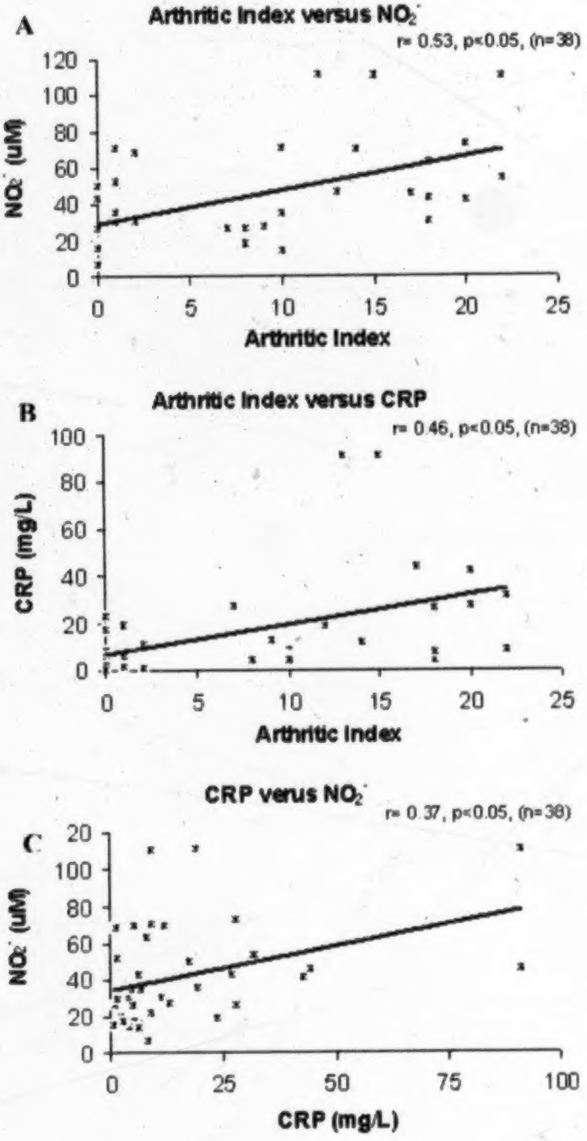


Figure 3-2: Correlation graphs between Arthritic Index and CRP (A), Arthritic Index and NO$_2^-$ (B), and CRP and NO$_2^-$ (C).
Pearson's correlation coefficient was used to calculate significance at $\alpha = 0.05$.

Valsartan Pharmacokinetics

Valsartan pharmacokinetics in each group was determined from the plasma concentration versus time graphs. There was no significant difference in the drug pharmacokinetics in presence of inflammation. Indeed, the concentration-time profiles of valsartan were remarkably consistent among the three groups (Figure 3-3, Table 3-3). Protein binding of valsartan during inflammation was not determined in our study since no change in pharmacokinetics was observed.

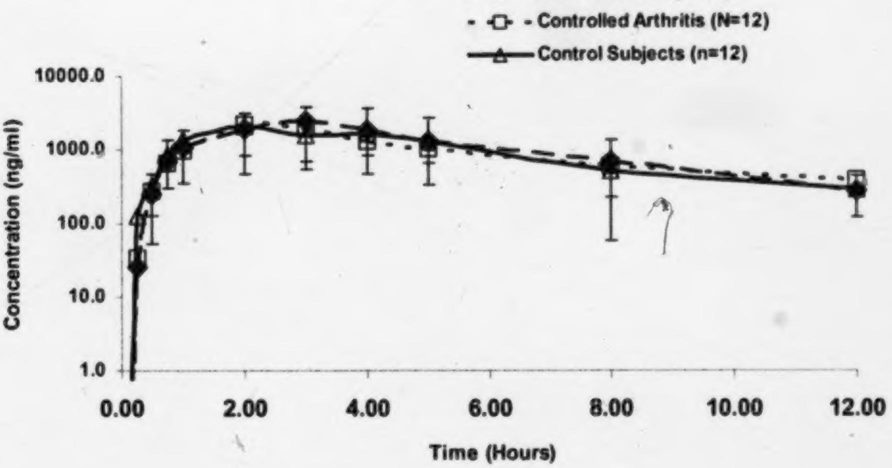


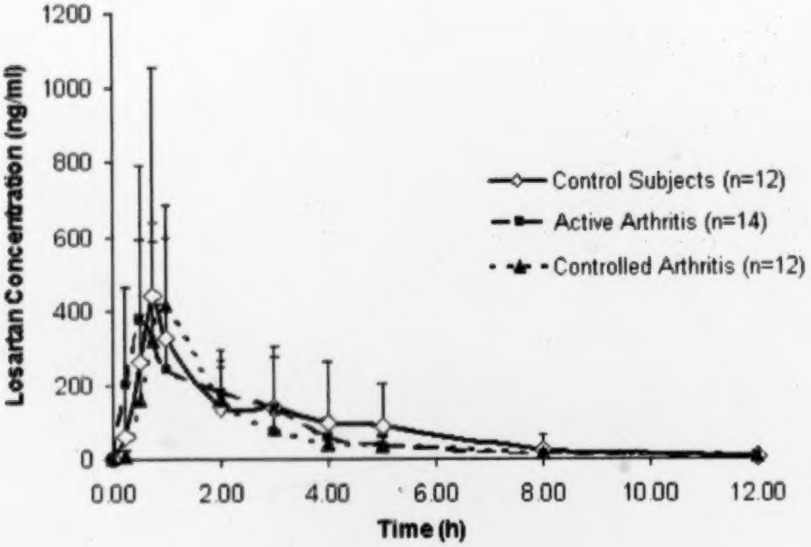
Figure 3-3: Mean ± SD of plasma valsartan concentration-time curves for healthy subjects, active arthritis and Controlled arthritic groups following oral administration of 160 mg.

Table 3-3: Mean ± SD valsartan pharmacokinetic indices.
Control (n=12), Active Arthritis (n=14), Controlled Arthritis (n=12).

	Tmax, h	Cmax, mg/L	$t_{1/2}$, h	AUC, mg.h/L	Vd/F, L	CL/F, L/h
Control	2.6 ± 1.0	2.4 ± 1.1	3.2 ± 1.1	12.9 ± 5.5	68.9 ± 40.8	16.6 ± 11.6
Active Arthritis	2.6 ± 0.7	2.6 ± 1.3	3.4 ± 0.9	13.8 ± 8.9	81.2 ± 46.2	16.1 ± 8.2
Controlled Arthritis	2.4 ± 0.9	2.4 ± 1.7	4.5 ± 3.5	14.3 ± 15.4	131 ± 121	18.7 ± 12.2

Losartan Pharmacokinetics

The concentration vs. time graphs of losartan and the active metabolite EXP 3174 are presented for the three subject groups in Figure 3-4. Rheumatoid arthritis did not significantly alter losartan pharmacokinetic parameters (Figure 3-5, Table 3-4). However, the presence of inflammation altered the pharmacokinetics of the major metabolite EXP 3174. In particular, the AUC of EXP 3174 was significantly lower in arthritic patients (Figure 3-5). The AUC ratio of EXP 3174/losartan also negatively correlated with disease severity ($r = 0.35$, $p < 0.05$, $n = 38$). No difference in the maximum concentration, terminal elimination half-life, or the time to maximum concentration of EXP 3174 was observed. Samples from one healthy subject (Figure 3-6) did not contain detectable concentrations of EXP 3174. The blood pressure response of this patient was within the range observed for other healthy subjects.



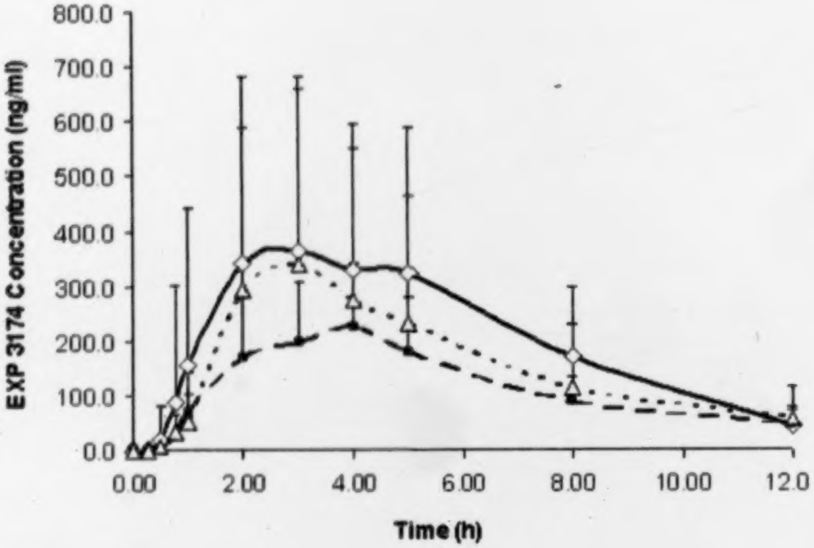


Figure 3-4: Mean ± SD of plasma concentration versus time curves of losartan (top) and EXP 3174 (bottom) for the three groups of subjects. Control (n=12), Active Arthritis (n=14), Controlled Arthritis (n=12)

Table 3-4: Mean ± SD of Losartan and EXP 3174 pharmacokinetic indices.

	Control Subjects (n=12)	Active Arthritis (n=14)	Controlled Arthritis (n=12)
Losartan			
Tmax (h)	1.4 ± 1.1	1.2 ± 0.9	1.0 ± 0.3
Cmax (mg/L)	0.5 ± 0.6	0.5 ± 0.3	0.5 ± 0.2
$t_{1/2}$ (h)	1.8 ± 0.7	1.8 ± 1.4	1.8 ± 0.9
AUC (mg.h/L)	1.1 ± 1.0	0.9 ± 0.4	0.9 ± 0.4
Vd/F (L)	369.7 ± 213,0	357.2 ± 324.1	327.5 ± 165.6
CLF (L/h)	182.7 ± 133.3	146.4 ± 88.0	136.6 ± 67.4
EXP 3174			
Tmax (h)	3.1 ± 1.1	3.1 ± 1.1	2.7 ± 1.1
Cmax (mg/L)	0.5 ± 0.3	0.3 ± 0.1	0.4 ± 0.2
$t_{1/2}$ (h)	5.7 ± 8.1	3.6 ± 1.2	5.0 ± 3.1
AUC (mg.h/L)	4.2 ± 4.3	1.7 ± 0.6*	2.4 ± 1.1*
AUC Ratio[a]	6.0 ± 6.8	2.3 ± 1.5	3.1 ± 2.1

[a] AUC Ratio EXP 3174 /LOSARTAN

*Significantly different from other groups (one way ANOVA followed by the Duncan's New Multiple Range test at $\alpha = 0.05$).

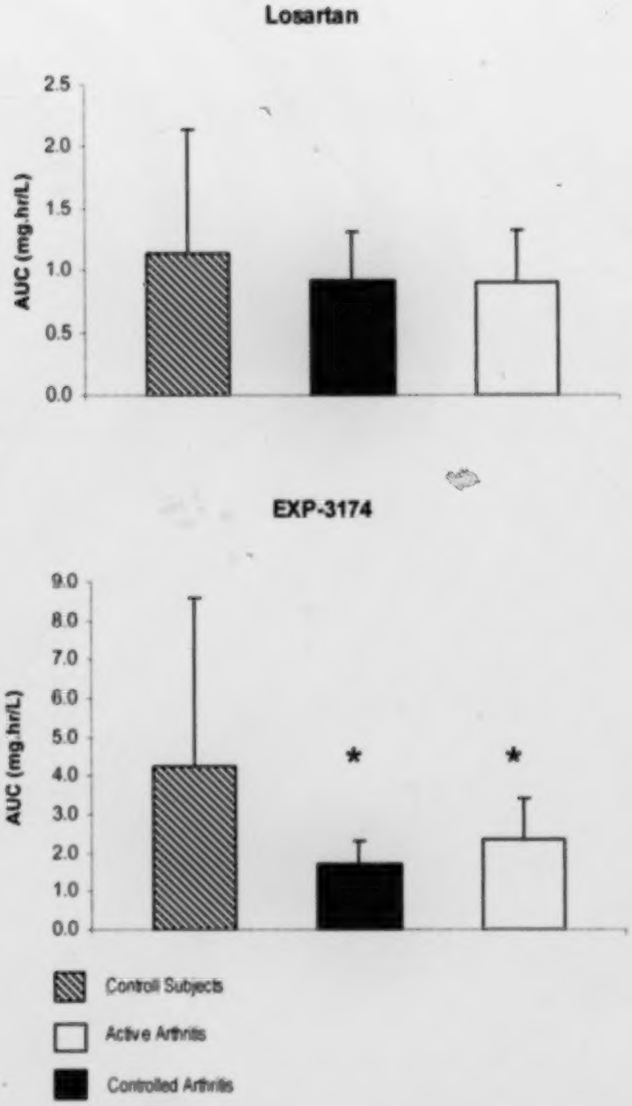


Figure 3-5: Graphical presentation of the Mean ± SD of AUC for losartan and EXP 3174 in control, active RA and Controlled RA subjects.
* Significantly different from other groups (one way ANOVA followed by the Duncan's New Multiple Range test at $\alpha = 0.05$). Control (n=12), Active Arthritis (n=14), Controlled Arthritis (n=12).

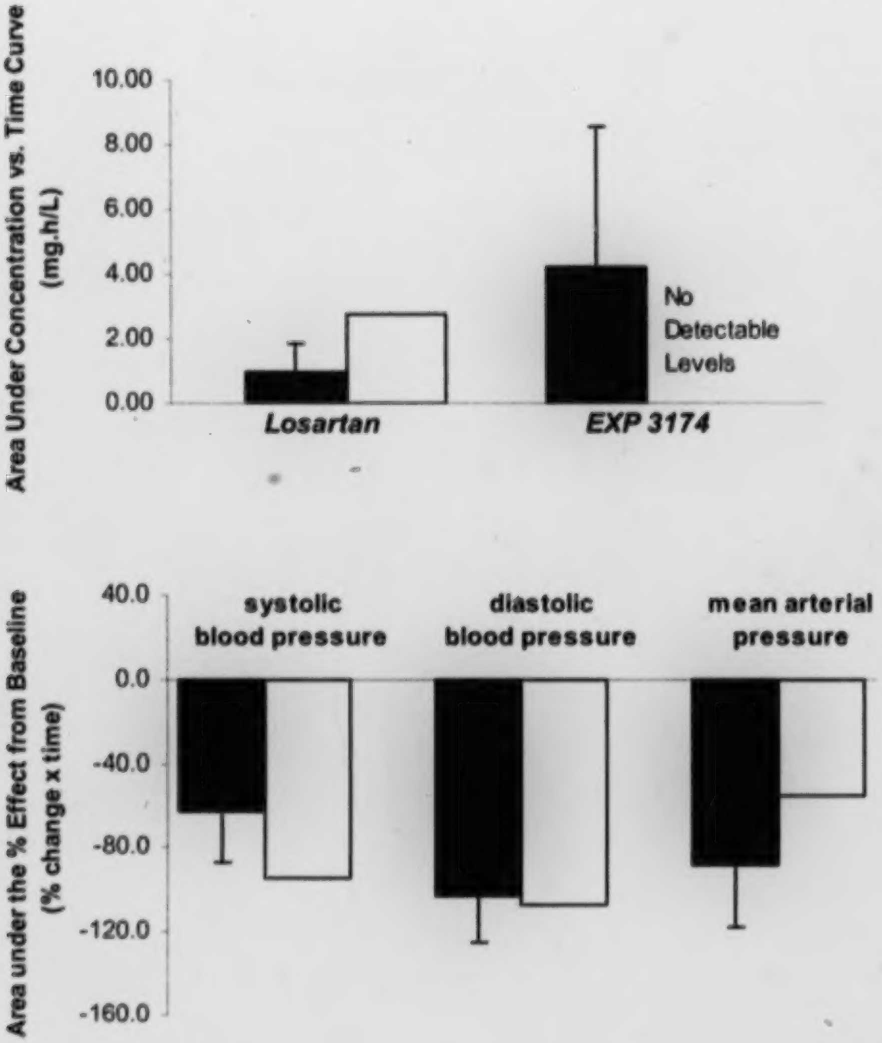


Figure 3-6: Abnormal subject Data. Area under the Concentration versus time (AUC) curve for losartan and EXP 3174 and the area under the percent effect from baseline x time curve (AUEC) in an abnormal (open bars) subject (n=1) compared to the mean of control populations (closed bars; n=11). Results are Mean ± SD for the AUC and Mean ± SEM for the AUEC. There were no detectable EXP 3174 levels in the plasma of the abnormal subject.

Pharmacodynamic Characteristics

We measured and analyzed 13 pharmacodynamic parameters among the study subjects. The area under the percent effect from baseline versus time (AUEC) and the maximum percent effect from baseline were analyzed for all the parameters. Table 3-5 and 3-6 represent pharmacodynamic changes observed with valsartan and Table 3-7 and 3-8 for losartan. However, the focus of the study was maintained on the systolic and the diastolic blood pressure and the mean arterial pressure with drug treatment, as the changes in blood pressure parameters encompass the total effect of AT_1R antagonists on the cardiac and vascular parameters. The concentration versus percent effect changes from baseline graphs for the SBP, DBP and MAP are presented in Figure 3-7 for valsartan, Figure 3-10 for losartan, and Figure 3-11 for EXP 3174.

Valsartan Pharmacodynamics

There was no statistically significant difference in the baseline pharmacodynamic indices among groups. Valsartan administration was associated with a significant drop in blood pressure in all groups (Figure 3-8). Valsartan effect on both DBP and MAP, expressed as percent maximum and AUEC, appeared to be greater in both arthritic groups compared to healthy subjects. The difference, however, failed to reach statistical significance. Interestingly, the concentration versus % effect from baseline indicates increased effect in DBP and MAP for the two arthritic groups (Figure 3-7). As the blood pressure indices decrease with increasing concentration, a counter-clockwise hysterisis is observed. No difference among groups was observed at each time point of measurement when the percent effect from baseline versus time was graphed for SBP, DBP and MAP

Table 3-5 Mean ± SEM calculated for each parameter.

Valsartan Pharmacodynamics as expressed in area under the effect curve of the change from baseline			
	Control (n=12)	**Active Arthritis (n= 14)**	**Controlled Arthritis (n= 12)**
Systolic blood pressure	-24.5 ± 21.6	-38.8 ± 23.3	-22.2 ± 31.7
Diastolic blood pressure	-30.6 ± 44.3	-65.4 ± 26.8	-71.6 ± 56.2
Mean arterial pressure	-18.9 ± 20.8	-56.1 ± 29.0	-54.9 ± 38.9
Pulse	6.8 ± 17.6	-30.7 ± 15.7	-33.2 ± 17.3
cardiac ejection time	-8.0 ± 13.0	-0.9 ± 10.5	0.7 ± 10.1
Stroke volume	-8.9 ± 23.1	21.6 ± 17.4	20.0 ± 19.3
Stroke volume index	-4.7 ± 23.1	20.6 ± 18.2	18.4 ± 17.6
Cardiac output	-18.5 ± 11.7	-11.8 ± 12.3	-13.2 ± 8.7
Cardiac index	-15.2 ± 12.0	-10.0 ± 12.9	-18.8 ± 8.8
Large artery elasticity	74.4 ± 90.3	73.3 ± 44.8	23.7 ± 47.3
Small artery elasticity	130.1 ± 188.7	161.9 ± 91.8	307.9 ± 119.7
Systemic vascular resistance	-27.4 ± 49.4	-37.1 ± 26.0	-37.8 ± 50.1
Total vascular impedance	78.6 ± 143.9	12.7 ± 50.0	38.4 ± 40.5

Table 3-6: Mean ± SD calculated for each parameter.

Maximum effect seen with Valsartan treatment as expressed as % change from baseline			
	Control (n=12)	**Active Arthritis (n= 14)**	**Controlled Arthritis (n= 12)**
Systolic blood pressure	-9.8 ± 5.6	-10.3 ± 7.4	-8.5 ± 12.9
Diastolic blood pressure	-9.7 ± 12.9	-13.3 ± 8.8	-15.2 ± 18.5
Mean arterial pressure	-9.3 ± 6.3	-14.6 ± 10.1	-13.6 ± 12.5
Pulse	1.5 ± 12.3	-2.1 ± 11.7	-7.3 ± 11.1
cardiac ejection time	-3.9 ± 5.2	-6.8 ± 6.3	-3.4 ± 3.5
Stroke volume	9.0 ± 11.2	5.2 ± 7.7	8.2 ± 6.6
Stroke volume index	9.5 ± 10.9	5.2 ± 9.6	8.3 ± 7.0
Cardiac output	2.5 ± 4.2	5.2 ± 7.7	8.2 ± 6.6
Cardiac index	2.4 ± 4.2	3.2 ± 8.2	1.5 ± 2.5
Large artery elasticity	34.0 ± 40.7	26.9 ± 22.3	23.0 ± 21.1
Small artery elasticity	65.3 ± 96.3	55.1 ± 25.5	67.9 ± 50.8
Systemic vascular resistance	-11.1 ± 13.8	-11.6 ± 13.0	-11.6 ± 18.8
Total vascular impedance	-13.9 ± 31.0	-16.2 ± 19.0	-10.2 ± 25.0

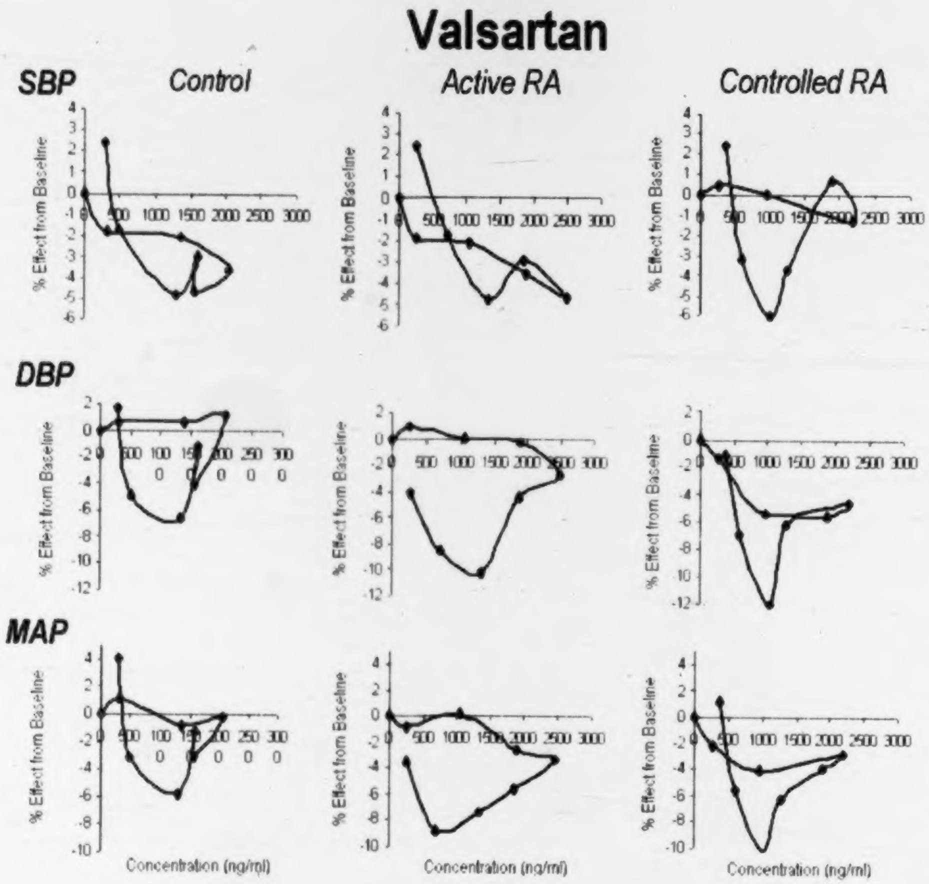


Figure 3-7: Concentration versus % effect from baseline curves for SBP, DBP and MAP with Valsartan treatment. Values represent Mean of the subjects within each group

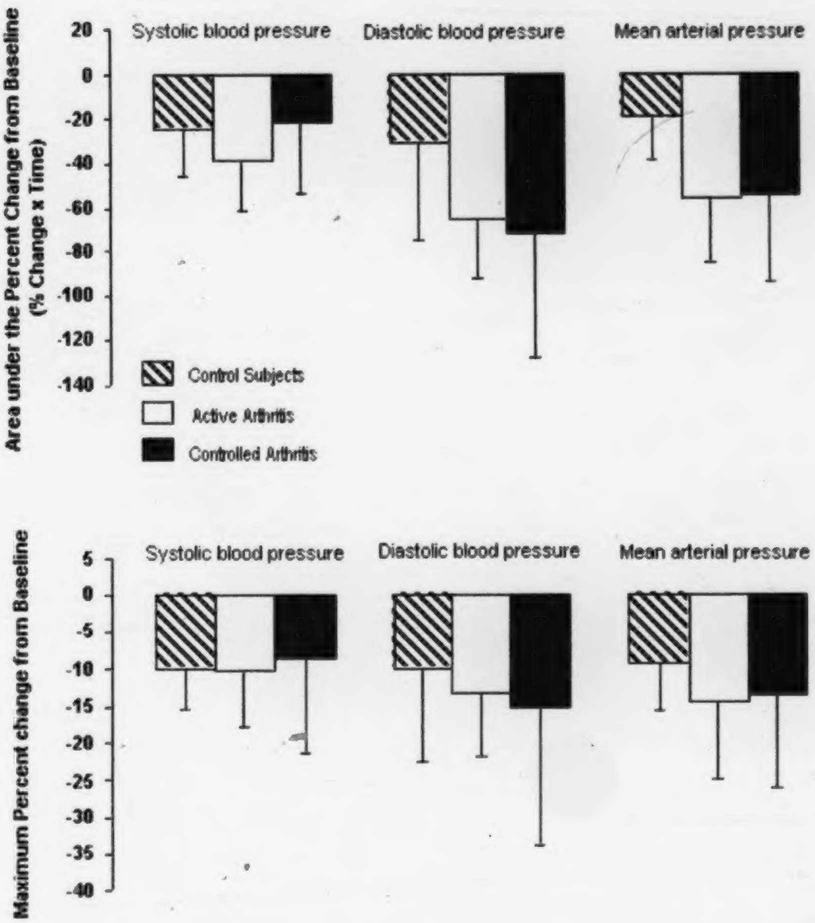


Figure 3-8: Effect of a single 160 mg oral dose of valsartan on blood pressure. Bars represent Mean ± SEM for AUEC and Mean ± SD for Maximum % change in effect from baseline. Control (n=12), Active Arthritis (n=14), Controlled Arthritis (n=12).

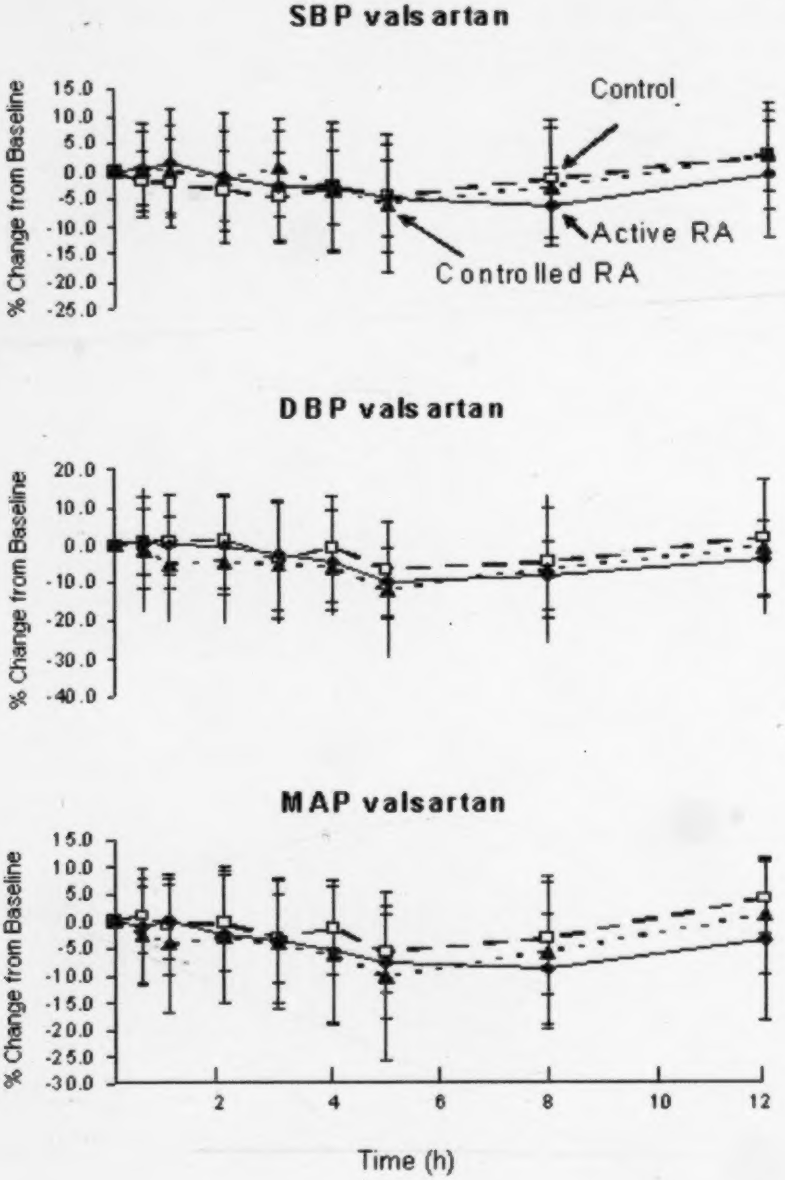


Figure 3-9: Percent change in effect from baseline versus time for SBP, DBP and MAP with valsartan dosing (160 mg) for control, active arthritis and controlled arthritis groups. Results are presented as Mean ± SD of Control (n=12), Active Arthritis (n=14), Controlled Arthritis (n=12) per time point.

(Figure 3-9). Similarly no significant difference was observed among the groups in terms of the effect of valsartan on other measured cardiovascular parameters, expressed as AUEC and maximum effect from baseline as presented in Table 3-5 and 3-6.

Losartan Pharmacodynamics

Losartan administration was associated with a significant drop in blood pressure in all groups (Figure 3-12). However, there was no statistically significant difference in the baseline pharmacodynamic indices among groups. No difference among groups was observed at each time point of sampling when the percent effect from baseline versus time was measured for SBP, DBP and MAP (Figure 3-13). The effect on SBP, DBP and MAP expressed as AUEC appeared to be much less in both arthritic groups than healthy subjects (Figure 3-12). The difference, however, failed to reach statistical significance. Similar lower response is seen with increasing concentrations of EXP 3174 in arthritic subjects, when the concentration versus % effect from baseline is determined for SBP, DBP and MAP (Figure 3-11). A counter-clockwise hysteresis is apparent when the concentration versus % effect from baseline is observed with both increasing losartan and EXP 3174 concentration (Figure 3-10, 3-11). Interestingly, there is an immediate drop in blood pressure with losartan administration, followed by further antihypertensive actitivity as the EXP 3174 level increase, indicating the total blood pressure lowering activity is attributed to both the parent drug as well as the active metabolite. The maximum percent change in blood pressure did not indicate any difference among subjects post drug administration. The AUEC and the maximum effect with losartan

Table 3-7: Mean ± SEM represented.

Losartan Pharmacodynamics as expressed in area under the effect curve of the change from baseline			
	Control (n=12)	**Active Arthritis (n= 14)**	**Controlled Arthritis (n= 12)**
Systolic blood pressure	-65.1 ± 22.9	-22.0 ± 29.4	-84.1 ± 20.3
Diastolic blood pressure	-103.7 ± 19.6	-53.4 ± 25.1	-112.4 ± 22.6
Mean arterial pressure	-85.5 ± 27.3	-61.4 ± 25.9	-99.0 ± 29.8
Pulse	0.4 ± 16.7	-14.7 ± 14.5	-43.8 ± 18.8
cardiac ejection time	-6.2 ± 13.8	-21.3 ± 11.1	5.8 ± 13.5
Stroke volume	0.4 ± 20.9	-15.1 ± 11.4	40.0 ± 22.8
Stroke volume index	-1.3 ± 21.0	-15.3 ± 12.3	44.0 ± 21.4
Cardiac output	-15.1 ± 20.2	-27.6 ± 13.4	-25.1 ± 13.9
Cardiac index	-12.7 ± 17.9	-24.0 ± 14.6	-27.6 ± 15.1
Large artery elasticity	193.3 ± 101.2	53.3 ± 65.5	230.5 ± 53.9
Small artery elasticity	198.0 ± 131.6	299.4 ± 124.2	198.9 ± 103.0
Systemic vascular resistance	-76.1 ± 40.2	-13.2 ± 24.2	-76.4 ± 24.8
Total vascular impedance	-72.5 ± 93.6	17.6 ± 59.4	-108.0 ± 29.0

Table 3-8: Mean ± SD represented.

Maximum effect seen with Losartan treatment as expressed as % change from baseline			
	Control (n=12)	**Active Arthritis (n= 14)**	**Controlled Arthritis (n= 12)**
Systolic blood pressure	-11.5 ± 8.3	-11.2 ± 8.5	-14.2 ± 4.7
Diastolic blood pressure	-15.9 ± 7.5	-13.7 ± 9.3	-18.7 ± 8.3
Mean arterial pressure	-16.9 ± 7.3	-14.4 ± 8.4	-18.7 ± 8.4
Pulse	-3.2 ± 12.1	-1.9 ± 12.6	-7.3 ± 12.9
cardiac ejection time	-5.4 ±6.8	-3.5± 7.0	-3.1 ± 4.8
Stroke volume	8.4 ±7.3	5.0 ± 9.6	11.9± 10.9
Stroke volume index	8.4 ± 7.4	5.0 ± 10.2	12.1 ± 11.1
Cardiac output	3.6 ± 7.0	2.9 ± 7.2	3.2 ± 4.8
Cardiac index	4.0 ± 6.8	2.4 ± 7.6	2.9 ± 5.3
Large artery elasticity	36.7 ± 34.1	29.2 ± 33.5	43.7 ± 20.4
Small artery elasticity	67.9 ± 59.3	80.5 ± 92.2	78.6 ± 65.6
Systemic vascular resistance	-14.3± 11.5	-12.1 ± 11.1	-16.2 ± 6.9
Total vascular impedance	-25.6 ± 26.1	-19.7 ± 18.2	-25.9 ± 7.9

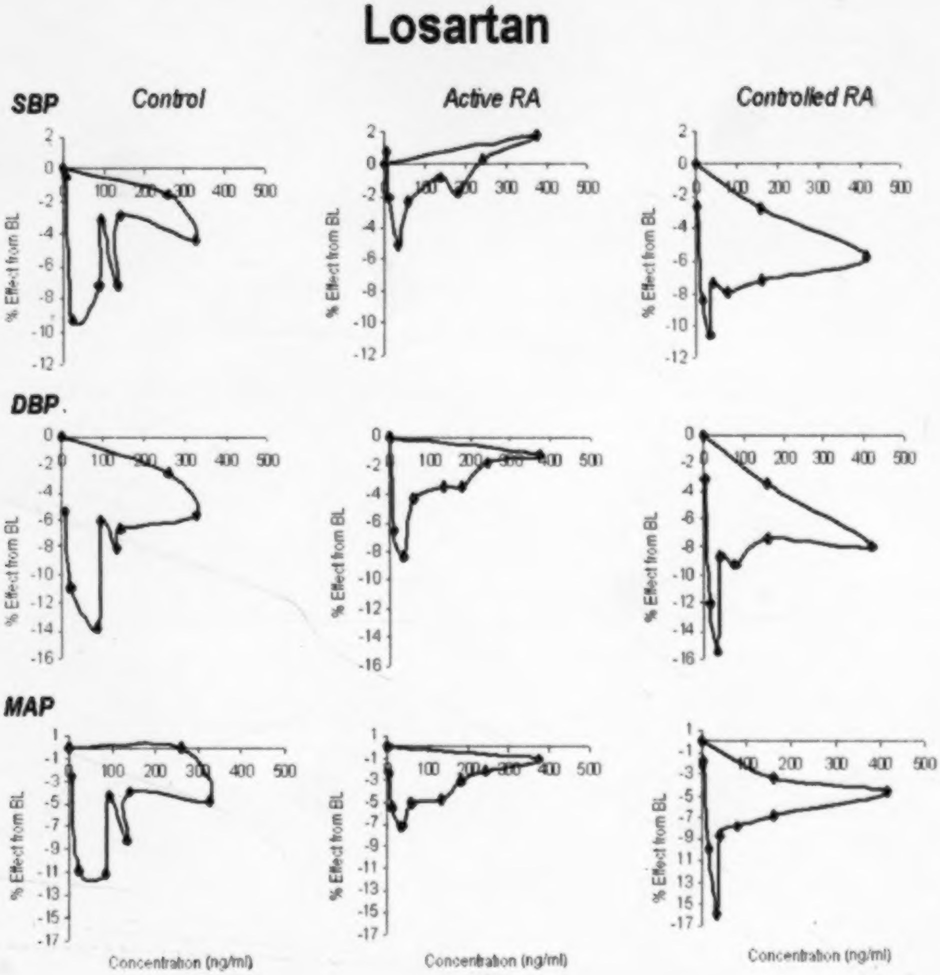


Figure 3-10: Concentration versus % effect from baseline curves for SBP, DBP and MAP with Losartan treatment. Values represent mean of the subjects within each group per point. Control (n=12), Active RA (n=14), Controlled RA (n=12).

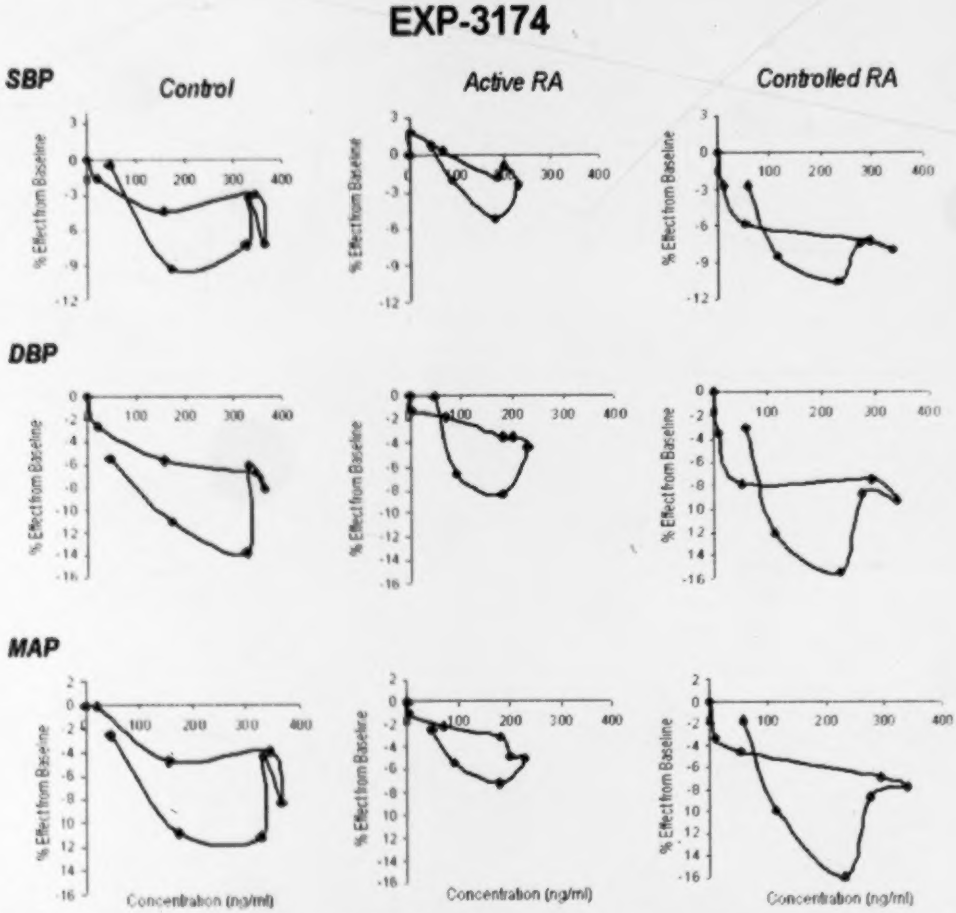


Figure 3-11: Concentration versus % effect from baseline curves for SBP, DBP and MAP with EXP 3174 concentration values. Values represent mean of the subjects within each group per point. Control (n=12), Active RA (n=14), Controlled RA (n=12).

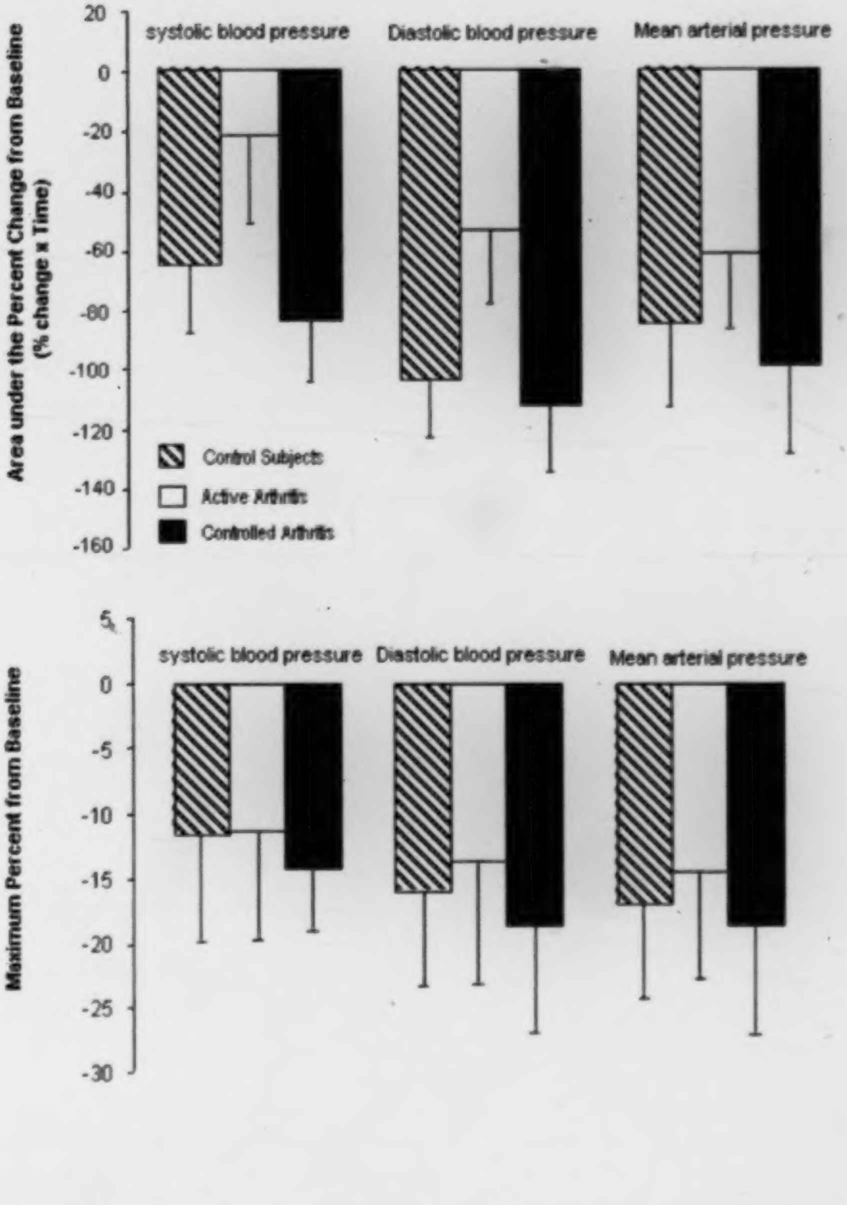


Figure 3-12: Effect of a single 100 mg oral dose of losartan on blood pressure. Bars represent Mean ± SEM for AUEC and SD for maximum % change in effect from baseline. Control (n=12), Active Arthritis (n=14), Controlled Arthritis (n=12).

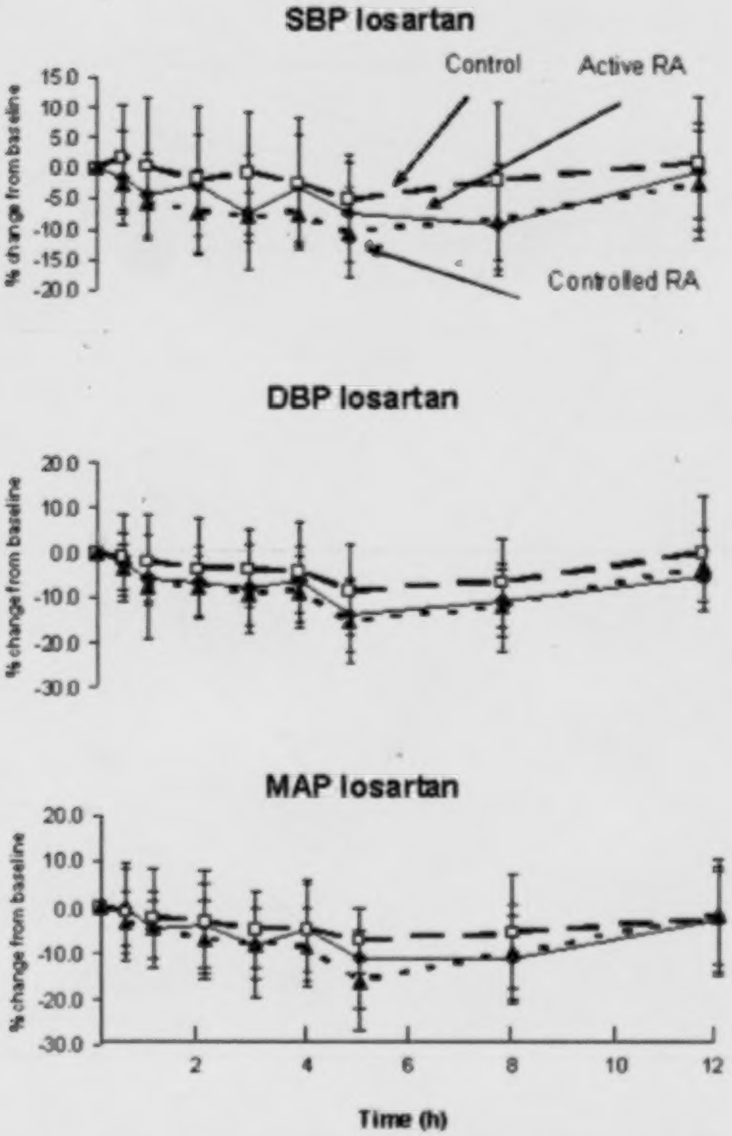


Figure 3-13: Percent change in effect from baseline versus time for SBP, DBP and MAP with losartan dosing (100 mg) for control, active arthritis and controlled arthritis groups. Results are presented as Mean ± SD. Control (n=12), Active Arthritis (n=14), Controlled Arthritis (n=12) per time point.

dosing for other cardiovascular indices also did not show any significant difference among the groups, as expressed in Table 3-7 and 3-8.

Discussion:

Inflammatory states have been shown to alter the pharmacokinetics and pharmacodynamics of various cardiovascular drugs including β AR antagonists and Ca^{2+} channel blockers (Kulmatycki et al., 2001; Mayo et al., 2000; Guirguis et al., 2003). It has been shown that the calcium channel blocker verapamil has decreased potency in prolonging the PR interval in normotensive rheumatoid arthritis patients as compared with healthy subjects despite increased plasma concentrations (Mayo et al., 2000). Using animal models of inflammation, similar altered pharmacodynamics have been reported for verapamil as well as for the β-adrenergic blockers propranolol and sotalol (Guirguis et al., 2003; Kulmatycki et al., 2001). Such findings are therapeutically relevant since these drugs are used in the treatment of such cardiovascular diseases such as myocardial infarction (MI), atherosclerosis and angina pectoris. As in the case of rheumatoid arthritis, the latter conditions are also associated with increased expression of pro-inflammatory mediators. There are significant increases in TNFα, interleukin 1β, interleukin 6, CRP and NO in MI, essential hypertension and angina (Balbay et al., 2001; Carlstedt et al., 1997; Sung et al., 2003; Rosenson et al., 2002; Deten et al., 2002). Our results demonstrate an average 2-3 fold increase in plasma NO_2^-, a stable breakdown product of NO, as well as up to 4 fold increases in CRP levels (Figure 3-1, Table 3-2) associated with active RA.

The observed changes in pharmacodynamics of these cardiovascular drugs in inflammation may have important therapeutic consequences (Hinz et al., 1975). Rheumatoid arthritis patients have an increased mortality rate (Prior et al., 1984; Mutru et al., 1985) and die on average 2.5 years earlier in community-based studies (Myllykangas-Luosujarvi et al., 1995) and up to 18 years in hospital-based cohorts (Wolfe et al., 1994). Approximately 50% of these deaths are due to cardiovascular diseases (Wolfe et al., 1994). The reason for this increased cardiovascular mortality in RA is not clear. However, in addition to the generally acknowledged risk factors (i.e., hypertension, smoking, high cholesterol and obesity), elevated baseline diastolic blood pressure and a prothrombotic state have been suggested for arthritic patients (McEntegart et al., 2001; Wallberg-Jonsson et al., 2000). Very recently, in other forms of inflammation, namely MI, atherosclerosis and unstable angina, the presence of pro-inflammatory mediators such as CRP, TNFα and IL 6 have been identified as additional risk factors (Griselli et al., 1999; Deten et al., 2003; Pietila et al., 1996; Lindmark et al., 2001) Indeed, there appears to be a close association between death and elevated CRP after myocardial infarction (Pietila et al., 1996). Therefore, it is reasonable to question the influence of pro-inflammatory mediators in the high mortality rate in both RA and cardiovascular patients. This is particularly important since based on the limited human data (Mayo et al., 2000), supported by observations made using experimental animals (Kulmatycki et al., 2001; Sattari et al., 2002) and *in vitro* tests (Penn et al., 1996; Hammond et al., 1992; Bernardin et al., 1998; Kadoi et al., 2002), the presence of these mediators also appear to reduce the potency of some drugs used in the treatment of cardiovascular diseases.

Reduced potency of cardiovascular drugs may, at least in part, be due to a down-regulation and/or inactivation of the receptors and channels, caused by inflammatory mediators. In fact, a decrease in β adrenergic receptor density and binding sites in the airway, heart, as well as blood mononuclear cells have been observed with increase in inflammation (Penn et al., 1996; Hammond et al., 1992; Bernarin et al., 1998; Kadoi et al., 2002; Krause et al., 1995). Recent observations made using a rat model of inflammation suggest the down regulation of the myocardial β_1 adrenoceptor, both at the level of receptor binding (Sattari et al., 2003) and density (Sattari et al., 2002). Altered receptor function may also be due to receptor uncoupling of β adrenoceptor from guanine nucleotide protein or intracellular modifications to protein kinases, adenylate cyclase activity and cAMP regulation and changes in calcium trafficking (Penn et al., 1996; Hammond et al., 1992; Bernardin et al., 1998; Kompa et al ., 1999).

The downregulations in effect observed were with the G-protein coupled receptor beta adrenoceptor blockers and calcium channel blockers. Although AT_1Rs are also G-protein coupled receptors (Thomas et al., 1999), their activation and signal transduction mechanism, as well as their intracellular messengers, varies somewhat from β adrenoceptor (Sayeski et al., 1998). We therefore did not expect similar results to be seen with AT_1R antagonists. With the emergence and increase in the usage of angiotensin II receptor antagonists as a treatment option for cardiovascular diseases, mainly hypertension and CHF (Toth et al., 2003), it became important to understand the effect of inflammation on the pharmacokinetic and pharmacodynamics of these drugs.

Our present data suggest that inflammation, at least, as manifest in RA, has no down-regulating effect on AT_1R antagonist valsartan. This is contrary to the reduced response reported for verapamil (Mayo et al., 2000) in humans as well as propranolol (Guirguis et al., 2003) and sotalol (Kulmatycki et al., 2001) in experimental animals. Indeed, patients with RA demonstrated a trend toward increased response to the hypotensive effect of valsartan as compared to the control group (Figure 3-6). The apparent increased potency did not reach statistical significance due, perhaps, to the limited study population size and high variability in response. However, increasing evidence regarding the effect of CRP and TNFα in up-regulating AT_1Rs in various inflammatory conditions including atherosclerosis, myocardial infarction and congestive heart failure may shed more light in valsartan effect in RA subjects (Gurantz et al., 1999; Kaprielian et al., 1997; Peng et al., 2002; Wang et al., 2003; de Boer et al., 2003). For example, increased levels of TNFα and interleukin 1β augment the mRNA levels of the AT_1R by up to 5-fold in cardiac fibroblasts (Gurantz et al., 1999). Studies with insulin growth factor indicate a post-transcriptional mechanism, leading to mRNA stabilization. There is also an up-regulation of the renin-angiotensin system during differentiation of monocytes to macrophages (Okamura et al., 1999), and increased angiotensin II levels in MI and LVH (Gurantz et al., 1999). The above-mentioned changes, combined with increase in AT_1R expression, may exaggerate the vascular response via AT_1R on cardiovascular parameters (Tanaka et al., 1999). Although the mechanisms of up-regulation have not been fully elucidated, some studies have looked into the possibility of the involvement of PKC and tyrosine kinases in the up-regulation mechanism (Motojima et al., 1999).

There was also a pronounced decrease in pulse, indicative of decreased heart rate and function with valsartan and losartan treatment in RA patients as seen in Table 3-5 to Table 3-8. It was expected that there would be a compensatory increase in heart function with a drop in blood pressure with the use of AT_1R antagonists, which would lead to an increase in pulse, through increasing adrenergic activity. Since heart rate is in control of β_1 AR, the result was not explicable, until recent results by Barki-Harrington et al suggested a possible heterodimerization of AT_1R with β_1 AR receptors, and a resulting cross-talk between the two receptor types, as expressed by dual inhibition of β_1 AR and AT_1R by AT_1R antagonist valsartan (Barki-Harrington et al., 2003). The trans-inhibitory effect of both receptors by one single antagonist may occur due, possibly, to valsartan interference with β AR-G*s* coupling. As a result, the downstream signaling and trafficking of β-AR is effectively reduced by valsartan treatment. This, coupled with the potential up-regulation in AT_1R level in the cardiac myocytes of RA patients, would explain the compounded inhibitory effect in the β-adrenergic receptors observed with AT_1R antagonist treatment in RA patients on the β-adrenergic receptor.

The increase in the AT_1R combined with a stable pharmacokinetic profile would explain the possible increase in potency of valsartan in controlling blood pressure in RA subjects who have an increased level of inflammatory mediators. As well, AT_1R blockade would increase the level of AGII, which is also increased in inflammatory state (Phillips et al., 2002). The AGII would then exert its effect via the AT_2R, which would potentiate further vasodilation (Carey et al., 2001). This attribute of valsartan would be useful for patients who may exhibit an inadequate control of their cardio-inflammatory disorders with their prescribed β-AR antagonists and calcium channel blockers. This leads to the potential therapy failure and high mortality rate due to secendary cardiovascular complications with their RA.

An unexpected finding was the apparent lag time between valsartan dosing and antihypertensive effect, leading to the counter-clockwise hysterisis in the concentration versus percent effect from baseline graphs (Figure 3-7). A counterclockwise hysterisis is often seen when a prodrug is administered, as there is a time delay between the formation of the active metabolite and the exerted effect. Since valsartan is in itself the active compound, this reasoning can be negated. Interestingly, the counterclockwise hysterisis is also evident with losartan dosing (Figure 3-10). Comparison of the active metabolite EXP 3174 concentration to the observed effect was expected to collapse the hysterisis and linearize the graph. Surprisingly, we also observe an unexpected time lag in effect with the active metabolite (Figure 3-11). The possible explanation for the time lag in effect may be due to the need for AT_1R antagonists to travel into another compartment in order to exert their actions on the receptor. Although AT_1R preside in many organs, including the brain, kidney, heart and blood vessels, the exact location of the receptors within the tissue and the organ have not been properly characterized. Further work must be done in order to characterize the AT_1R availability to its effectors.

Valsartan is mainly eliminated through biliary excretion (~70%) with metabolism contributing only to approximately 20% of its clearance (Waldmeier et al., 1997; Müller et al., 1997). This limited dependency of clearance on metabolism may explain the observed lack effect by RA on valsartan pharmacokinetics. It is mainly drugs with efficient hepatic clearance that have been shown to be influenced by inflammation (Piquette-Miller et al., 1995). However, as biliary excretion is the major pathway involved in the removal of valsartan, we must consider the effect of inflammation on this pathway as well. Recent studies have shown an increase in methotrexate concentration in adjuvant arthritic rats due to a decrease in biliary excretion. More specifically, adjuvant arthritis decreased the expression and activity of the hepato-biliary ATP-dependent transport system cMOAT, or MRP2, involved in methotrexate elimination (Achira et al., 2002). Valsartan is thought to use the same transporter system for biliary excretion, although the importance of the transporter in valsartan's biliary excretion has not been fully elucidated. Our human study has shown the lack of pharmacokinetic changes in valsartan. Possibly, the level of inflammation in our subjects was less severe and therefore MRP2 is not altered. However, the possibility of pharmacokinetic drug interaction for MRP 2 between methotrexate and valsartan if taken concurrently may be considered if adverse effects are noted.

Valsartan is over 92% bound to plasma albumin. Active arthritis is associated with decreased albumin (Van Den Ouweland et al., 1988).This may result in enhanced clearance due to reduced plasma protein binding. This was not observed in our patients. Indeed, the plasma-concentration-time curves of the three examined groups were remarkably close (Figure 3- 2) and in agreement with those observed previously (Müller el al., 1997). A change in the plasma protein binding and subsequently clearance of drugs has been observed only with naproxen and in patients with severe arthritis. In other words, with patients with CRP concentrations more than double of what we recorded for our patients, changes in clearance is inevitable (Van Den Ouweland et al., 1988).

Thus, inflammation as manifest in rheumatoid arthritis does not alter the pharmacokinetics of valsartan. Similarly, the pharmacodynamic response to valsartan is not down regulated by inflammation either. This class of drugs should therefore be taken into consideration in treating cardiovascular conditions in the setting of inflammation, for there is no decrease in their effectiveness seen as compared to calcium channel blockers or β-blockers.

To observe whether the effect seen with valsartan can be pronounced as a class effect and thus generalized to include AT_1R antagonists with complicated pharmacokinetics, we looked at losartan, one of the first orally active non-peptide AT_1R antagonists to be made available on the market. Losartan is nearly completely absorbed, but undergoes extensive first pass metabolism (McIntyre et al., 1997). As previously mentioned, although losartan itself is an active drug, its potency is mainly attributed to the active carboxylic acid metabolite EXP 3174 (Lo et al., 1995). Approximately 14% is converted to EXP 3174 by CYP 2C9 and 3A4, and about 80% is converted into other metabolites (McIntyre et al., 1997; Lo et al., 1995). Since hepatic metabolism is known to be inhibited by inflammation and the presence of increased pro-inflammatory mediators, we hypothesized decreased bioavailability for EXP 3174, and thereby a reduced efficacy, in RA patients.

Losartan is recommended over certain β blockers in the treatment of isolated systolic hypertension and left ventricular hypertrophy, all diseases that exhibit increases in inflammatory mediator levels (Toth et al., 2003; Okin et al., 2003; Kjeldsen et al., 2002; Lindholm et al., 2002). Increases in NO and cytokines, as seen in rheumatoid arthritis subjects (Table 3-2), can alter the absorption and metabolism of drugs by changing Pgp and selective enzyme expression and levels (Sukhai et al., 2000; Achira et al., 2002; Bertilsson et al., 2001; Piquette-Miller et al., 2000; Iber et al., 1999; Jover et al., 2002.; Khatsenko et al., 1998; Renton et al., 2000; Morgan et al., 1997). Therefore, our goal was to determine whether the degree of inflammation, and the level of inflammatory mediators as seen in our RA patients, alters the disposition and effect of orally administered losartan.

There was a significant decrease in the AUC of EXP 3174 and a numerical decrease in the Cmax, with no significant change in the losartan pharmacokinetics in patients with active arthritis (Figure 3-4, Table 3-4). Also, there was a decrease in AUC ratio of EXP/LOS, with increasing severity of disease. The severity of disease also correlates positively with increasing CRP and NO levels.

The observed pharmacokinetic changes of EXP 3174 may be attributed to several factors. Since the elimination half life of EXP 3174 is not affected by inflammation, the significant decrease in EXP 3174 AUC can be postulated to be due to the reduced extent of formation of the metabolite. Losartan conversion to EXP 3174 involve P450 enzymes CYP2C9 and CYP 3A4 (McIntyre et al., 1997; Lo et al., 1995; Stearns et al., 1995). The conversion of losartan to EXP 3174 via CYP 3A4 and 2C9 may be decreased. Morgan *et al* report selective CYP 450 downregulation *in vivo* and *in vitro* with different diseases and models of disease (Morgan et al., 1997). Studies have shown reduced clearance of antipyrine, hexobarbital and theophylline in human volunteers given low doses of LPS (Shedlofsky et al., 1994). Moreover, reduced clearance of the drugs correlated with the initial peak values of TNFα and interleukin 6. Differential regulation of P450 genes by NO and cytokines during inflammation have been postulated to lead to selective downregulation of many P450 due to repression of gene transcription, changes in RNA and/or protein turnovers or direct decrease in P450 catalytic activity (Iber et al., 1999; Jover et al., 2002.; Khatsenko et al., 1998; Shedlofsky et al., 1994).

A change in the absorption of losartan must also be a consideration for the possibility in the altered pharmacokinetics of EXP 3174 in RA. After an oral dose, intestinal factors, such as altered epithelial permeability, varying expression of drug metabolizing enzyme CYP3A4 and/or altered expression of multi-drug resistance counteractive drug transporters such as Pgp (Sukhai et al., 2000; Achira et al., 2002; Bertilsson et al., 2001). Losartan, but not EXP 3174, is a Pgp substrate (Soldner et al., 2000). Studies have shown that inflammation influences drug bioavailability by varying gut transport. For example, Bertilsson *et al* have shown an increase in Pgp expression in Caco 2 cells with cytokine treatment (Bertilsson et al., 2001). As well, there is increase in epithelial permeability. The increased permeability of the epithelial cell will likely facilitate the uptake of losartan across the gut barrier. However, increased Pgp expression seen with high cytokine levels would hamper and counteract the epithelial changes. The overall effect of gut absorption affecting losartan absorption in inflammation can therefore be negligible (Bertilsson et al., 2001).

Similar to valsartan, both losartan and EXP 3174 are also highly bound to albumin (~ 98% for EXP 3174 and ~92% with losartan) and may therefore be affected by plasma protein changes seen with inflammation. Acute arthritis is associated with decrease in albumin levels, which may result in enhanced clearance of highly protein bound drugs like naproxen due to reduced plasma protein binding (Van Den Ouweland et al., 1988). There was no change in the oral clearance of losartan, therefore there does not seem to be a great alteration in albumin levels.

Since the decrease in EXP 3174 levels was not reflected in the terminal half-life of the metabolite, we can also assume that EXP 3174 elimination was not altered. Indeed, increased clearance of naproxen seen in the study by Van Den Ouweland et al (1988) was only observed in severely arthritic patients, which is not the case with our patients.

Interestingly, the pharmacokinetic profile of both losartan and EXP 3174 seen with the rheumatoid arthritis patients is analogous to the effect seen with fluconazole treatment. Fluconazole, a selective CYP 3A4 and 2C9 inhibitor, decreased the formation of EXP 3174 and yet did not alter the pharmacokinetic profile of losartan (Kaukonen et al., 1998). Selective inhibition of CYP enzymes by cytokines may therefore inhibit losartan biotransformation to EXP 3174, yet not hinder alternate elimination pathways for losartan to alter its pharmacokinetics.

Selective biotransformation was further observed with one normal subject, who exhibited no detectable concentrations of the active metabolite in the plasma, while the parent drug AUC was the second highest amongst all the subjects in the study (Figure 3-5). Although complete inhibition of losartan conversion to EXP 3174 is achieved by dual inhibition of CYP 3A4 and 2C9, the latter isozyme is the one predominantly involved in this particular biotransformation (Yasar et al., 2002) . CYP 2C9 is subject to polymorphism due to isozyme specific mutations. In particular the CYP 2C9*3 variant allele has been observed in some individuals and population groups, leading to < 1% formation of EXP- 3174 from losartan (McCrea et al., 1999; Sekino et al., 2003). Genotyping for this particular subject is recommended given the lack of EXP 3174 levels, as there are many other important drugs, among which are phenytoin, S-warfarin, tolbutamide and non-steroidal anti-inflammatory drugs, whose metabolism would be decreased by CYP2C9*3 allele (Yasar et al., 2002).

Treatment efficacy is an integral part of patient therapy as it is essential to optimize treatment for the specific disease condition. However, inflammatory diseases such as arthritis, myocardial infarction and angina make it difficult to predict the effectiveness of cardiovascular drugs, and drug efficacy cannot be generalized to a specific class of drugs. As previously discussed, a significant decrease in verapamil potency in PR interval prolongation is observed in RA patients. The systolic and diastolic blood pressure of the arthritic patients was also decreased as compared to the healthy subjects, although not to a significant degree. Decrease in drug response in presence of inflammation is not limited only to the calcium channel blockers. Similar results with the blood pressure indices and electrocardiogram of the heart were observed in animal models of acute inflammation and arthritis with β- AR antagonist treatment.

Interestingly, with AT_1R antagonist valsartan, a numerical increase in the SBP, DBP and MAP was observed in arthritic patients as compared to the normal subjects. The same subject groups were treated with losartan and no significant difference among the groups was observed (Figure 3-12). A numerical decrease in the AUEC however was seen with the active arthritis group. However, it was consistent with the lower EXP 3174 levels observed with inflammation, and thus receptor down-regulation is an unlikely explanation for the observed effect. Although inflammation decreased the EXP 3174 level after losartan treatment in the arthritic patients, and ultimately decreased the AUEC for active arthritic group, it did not have a profound effect on the maximum observable effect. This maybe due to the short acting, yet still active, parent drug losartan. Indeed, it appears that losartan has a major role in blood pressure control particularly at the beginning of drug therapy. The concentration versus the percent effect from baseline for losartan (Figure 3-10) and EXP 3174 (Figure 3-11) makes apparent the immediate antihypertensive effect of losartan at low concentrations of the EXP 3174. Hence, losartan itself offers immediate blood pressure control, and is comparable to that seen with valsartan treatment (Figure 3-7). With increasing EXP 3174 concentrations, the antihypertensive effect shifts to the longer acting active metabolite, providing a more stable and potent control on the blood pressure, even when the plasma concentration of losartan itself diminished. Therefore, it appears that the therapeutic efficacy of losartan is uncompromised by the presence of inflammatory mediators by the combined efforts of losartan and EXP 3174. This renders losartan to be useful in the treatment of hypertension in patients with hyper-inflammatory diseases, such as myocardial infarction, and rheumatoid arthritis. Clinical studies indicate losartan over certain β blockers in the treatment of isolated systolic hypertension and left ventricular hypertrophy, all diseases that exhibit increases in inflammatory mediator levels (Kjeldsen et al., 2002; Okin et al., 2003; Toth et al., 2003).

In conclusion, increasing inflammatory mediators, as seen in RA, alters the conversion of extensively metabolized AT_1R antagonist losartan to its major active metabolite EXP 3174, most likely due to the inhibition of the metabolizing enzymes CYP 2C9 and 3A4. However, the decrease in AUC of EXP 3174 does not result in a decrease in response, due possibly to pharmacological effect of the parent drug and/or the AT_1R up-regulatory mechanisms of inflammatory mediators. As the pharmacodynamics of

AT_1R antagonists are not down-regulated by RA as compared with β-adrenergic receptor and calcium channel antagonists, this class of drugs may have a higher therapeutic efficacy in the treatment of hypertension in cardio-inflammatory conditions and other inflammatory states.

Our study had a few limitations. First, it was conducted after a single dose of AT_1R antagonist, while the drugs are usually intended for long-term therapy. However, it has been demonstrated that single dose studies predict chronic pharmacodynamic responses (Morgan JM et al., 1997; Fuchs et al., 2000). Secondly, all the subjects were normotensive. Nevertheless, although blood pressure reduction is proportionate to baseline levels, the drug's hypotensive effect can be studied, albeit with less of a magnitude of effect, in normotensive subjects (Ueda et al., 1998). Hence, our data must be considered as preliminary in nature. The beneficial effect of AT_1R antagonists in patients who are afflicted with both inflammatory conditions and impaired cardiovascular function remains to be studied using a larger population of patients.

Chapter 4: Strain Difference in Response to Sotalol. Results and Discussion

Results:

Inflammatory State

Blood cell counts at baseline and after the first and second injection of either $IFN_{\alpha 2a}$ (Inflamed) group or saline (control) were compared to determine the occurrence of acute inflammation for each strain. Segmented neutrophil counts were significantly increased in all inflamed strains of rats (Table 4-1). The baseline segmented neutrophil counts were significantly less in SDR as compared with other two strains of rats ($p = 0.002$, Figure 4-1). Attempts were made to measure plasma CRP, NO and TNFα concentrations. Unfortunately, however, problems with the assay kits generated at the supplier's end and consequent repeated freeze and thawing resulted in unreliable results. Protocols provided with the inflammatory kits (Biosource International, Helica Biosystems), as well as NO assays by Archer *et al*, (1995) suggest sample degradations occur upon subsequent multiple freeze-thaw cycles.

Table 4-1: Mean ± SD of percent segmented neutrophil counts after first and second injections of saline or $IFN_{\alpha 2a}$.

Treatment group				
% Segmented Neutrophil	*Baseline*	*1st* $IFN_{\alpha 2a}$ *injection*	*2nd* $IFN_{\alpha 2a}$ *injection*	*P value*
SDR (n=4)	9.3 ± 4.9 *	23.5 ± 7.9	26.8 ± 6.2	**0.009**
WKY (n=6)	16.6 ± 4.6 *	22.2 ± 9.5	29.4 ± 4.0	**0.03**
SHR (n=6)	22.3 ± 6.3 *	34.2 ± 4.0	34.7 ± 3.8	**0.00064**
Control group				
% Segmented Neutrophil	*Baseline*	*1st saline injection*	*2nd saline injection*	*P value*
SDR (n=4)	13.0 ± 3.2	10.3 ± 8.1	12.0 ± 8.7	0.9
WKY (n=6)	17.5 ± 7.0	17.5 ± 10.6	16.0 ± 8.4	0.76
SHR (n=5)	19.8 ± 4.6	20.5 ± 3.4	20.3 ± 5.6	1.0

* Significantly different from other groups (one way ANOVA followed by the Duncan's New Multiple Range test at $\alpha = 0.05$).

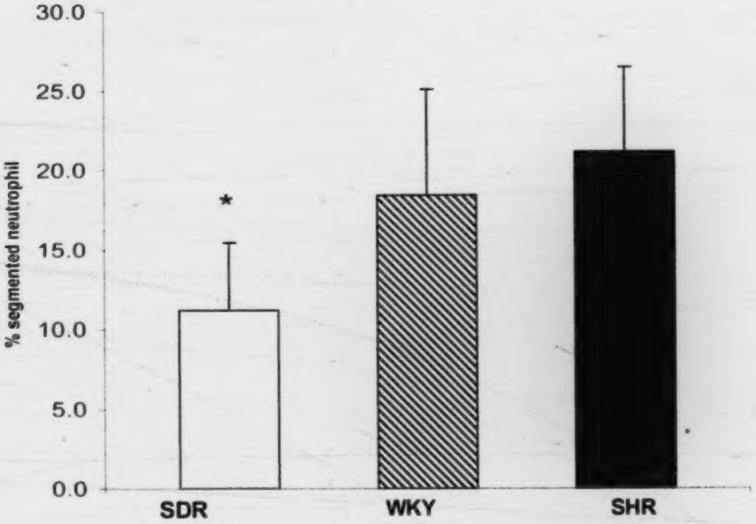


Figure 4-1: Mean ± SD of baseline % segmented neutrophil counts among the three strains.

* Significantly different from other groups (one way ANOVA followed by the Duncan's New Multiple Range test at $\alpha = 0.05$). SDR (n=8), WKY (n=12), SHR (n=11).

Pharmacodynamics

Effect of Interferon on ECG Parameters

Possible effect of $IFN_{\alpha 2a}$ itself on the rat ECG was determined by comparing the area under the percent effect change from baseline times time $(AUEC)_{0\text{-}3h}$ of RR, QT, QTc, and PR of the three strains after saline versus $IFN_{\alpha 2a}$ injection (Table 4-2). Inflammation induced by $IFN_{\alpha 2a}$ significantly increased the heart rate in the SDR, but did not affect other ECG parameters. As well, significant prolongation in the QT and QTc intervals was observed in the WKY strain with $IFN_{\alpha 2a}$ injection.

Effect of Sotalol on ECG Parameters

To observe sotalol effect in the prolongation of PR and QT intervals, the maximum effect (ECG parameters) following drug administration (Emax) were determined and compared to pre-sotalol dosing in control rats (Table 4-3). Sotalol significantly reduced the heart rate, as well as prolong the QT, QTc and PR intervals in the SDR strain, as expected. In WKY and SHR strains, sotalol induced a significant reduction in the QT and RR, similar to the SDR strain. However, sotalol treatment did not prolong the PR interval of WKY or SHR strains. Neither was there a significant response with regard to QTc prolongation in the SHR strain upon sotalol treatment.

Comparison of the Effect of Sotalol between Control and Inflamed groups

The effect versus time graph for sotalol, as well as the area under the effect curve (AUEC), was compared between control (saline) and inflammation ($\text{IFN}_{\alpha 2a}$) animals within each strain (Figure 4-2-Figure 4-6; Table 4-4). There was no difference between control and inflammation groups in their baseline ECG values prior to drug treatment. Treatment with sotalol reduced the heart rate in all strains regardless of inflammation. Sotalol effect on the QT and QTc prolongation was significantly reduced within the $\text{IFN}_{\alpha 2a}$ treated groups in the normotensive WKY and SDR strains. No difference was observed between the control and inflamed rats in the SHR with sotalol treatment. The ability of sotalol to prolong PR interval was also significantly decreased in the SDR strain. The lack of sotalol effect on PR interval prolongation in WKY or SHR strain was not altered with inflammation upon drug administration.

Strain Difference in ECG parameters

A statistically significantly faster baseline heart rate was observed in SHR as compared with the normotensive WKY and SDR strains (Table 4-5). None of the other ECG parameters were significantly different from each other.

When the response to sotalol of the three strains was compared, sotalol exhibited a significantly lower potency in the SHR strain with regards to the heart rate and the QT interval as compared to the SDR and WKY strains in the control group (Figure 4-7, Table 4-4). However, the difference failed to reach significance in the inflamed group.

Western Blot Analysis

In order to determine whether difference in sotalol response in PR interval prolongation is possibly due to strain differences in the β_1AR protein expression on the heart, western blot analysis was performed on whole heart proteins of control rats from hearts harvested from the rats one week after the study. No significant difference was observed in the β_1AR density between SDR, WKY, or SHR strains (Figure 4-5).

Table 4-2: Mean ± SD of AUEC $_{0\text{-}3h}$ for heart rate (RR), QT, PR and QTc intervals are determined after saline or IFN$_{\alpha 2a}$ injections.

AUEC $_{0\text{-}3\,h}$ Effect of interferon on ECG parameters (Area under the % change in effect from baseline x time)			
	Saline	**Interferon**	**P value**
RR			
SDR (n=4/group)	-29.0 ± 6.8	-1.3 ± 8.4	**0.002**
WKY (n= 6/group)	-2.9 ± 18.6	-7.6 ± 13.4	0.64
SHR (n=5-6/group)	36.7 ± 34.5	25.5 ± 22.6	0.52
PR			
SDR (n=4/group)	10.6 ± 14.3	9.0 ± 8.7	0.855
WKY (n= 6/group)	7.8 ± 8.6	7.2 ± 7.5	0.9
SHR (n=5-6/group)	-13.4 ± 24.1	-4.2 ± 8.3	0.41
QT			
SDR (n=4/group)	19.9 ± 10.2	17.5 ± 6.9	0.71
WKY (n= 6/group)	30.1 ± 29.4	58.8 ± 16.1	**0.0074**
SHR (n=5-6/group)	4.0 ± 19.3	30.8 ± 25.7	0.072
QTc			
SDR (n=4/group)	5.4 ± 8.6	12.5 ± 6.4	0.23
WKY (n= 6/group)	-11.5 ± 17.9	55.2 ± 16.8	**0.0001**
SHR (n=5-6/group)	19.6 ± 13.7	42.9 ± 28.5	0.11

*Significant difference between control and IFN$_{\alpha 2a}$ groups. (Student's t-test for unequal variance)

Table 4-3: Mean ± SD of the maximum % change from baseline in ECG parameters before (Placebo) and after (Treatment) sotalol treatment is determined among strains in the control group.

Maximum change in ECG parameters with Sotalol Dosing
(% Change from baseline)

	Placebo	Treatment	P value
RR			
SDR (n=4)	-14.6 ± 2.1	-24.4 ± 4	**0.01**
WKY (n= 6)	-7.7 ± 10.5	-21.4 ± 5.2	**0.02**
SHR (n=5)	-0.2 ± 14.8	-10.8 ± 3	0.15
PR			
SDR (n=4)	7.6 ± 6.7	17.9 ± 3.9	**0.046**
WKY (n= 6)	6.4 ± 3.9	6.8 ± 5.3	0.89
SHR (n=5)	-0.4 ± 8.4	1.6 ± 5.1	0.64
QT			
SDR (n=4)	10.6 ± 4.9	36.5 ± 11.1	**0.01**
WKY (n= 6)	17.9 ± 17.7	45.2 ± 10.2	**0.01**
SHR (n=5)	6.4 ± 6.3	31.2 ± 10.4	**0.001**
QTc			
SDR (n=4)	5.7 ± 4.2	21.5 ± 5.8	**0.01**
WKY (n= 6)	15.3 ± 10.2	30.0 ± 6.2	**0.02**
SHR (n=5)	13.7 ± 6.3	27.9 ± 9.2	**0.01**

* Significant difference between placebo and treatment (Student's t-test for unequal variance).

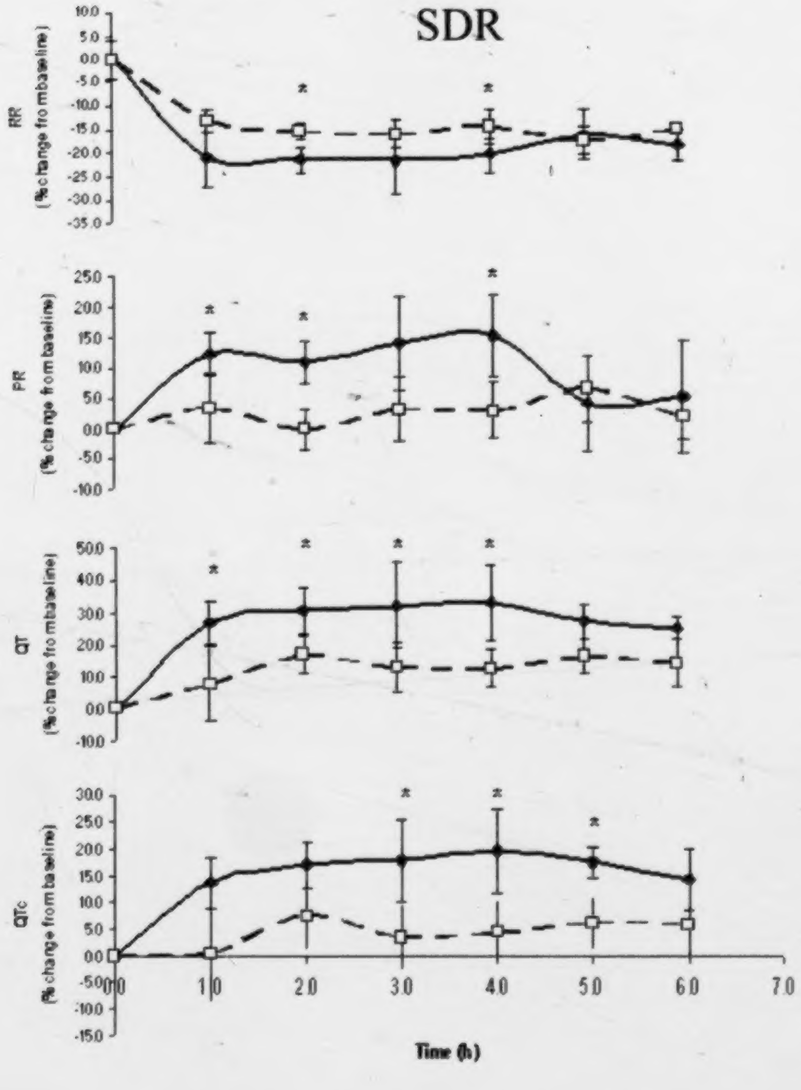


Figure 4-2: Mean ± SD of the time courses of sotalol % effect from baseline following administration of single oral doses of 80 mg/kg sotalol racemate to control (◆) and IFN$_{\alpha 2a}$ (□) treated rats. * Significant difference between control and IFN$_{\alpha 2a}$ (Student's t-test for unequal variance). Control (n=4) and Treated (n=4).

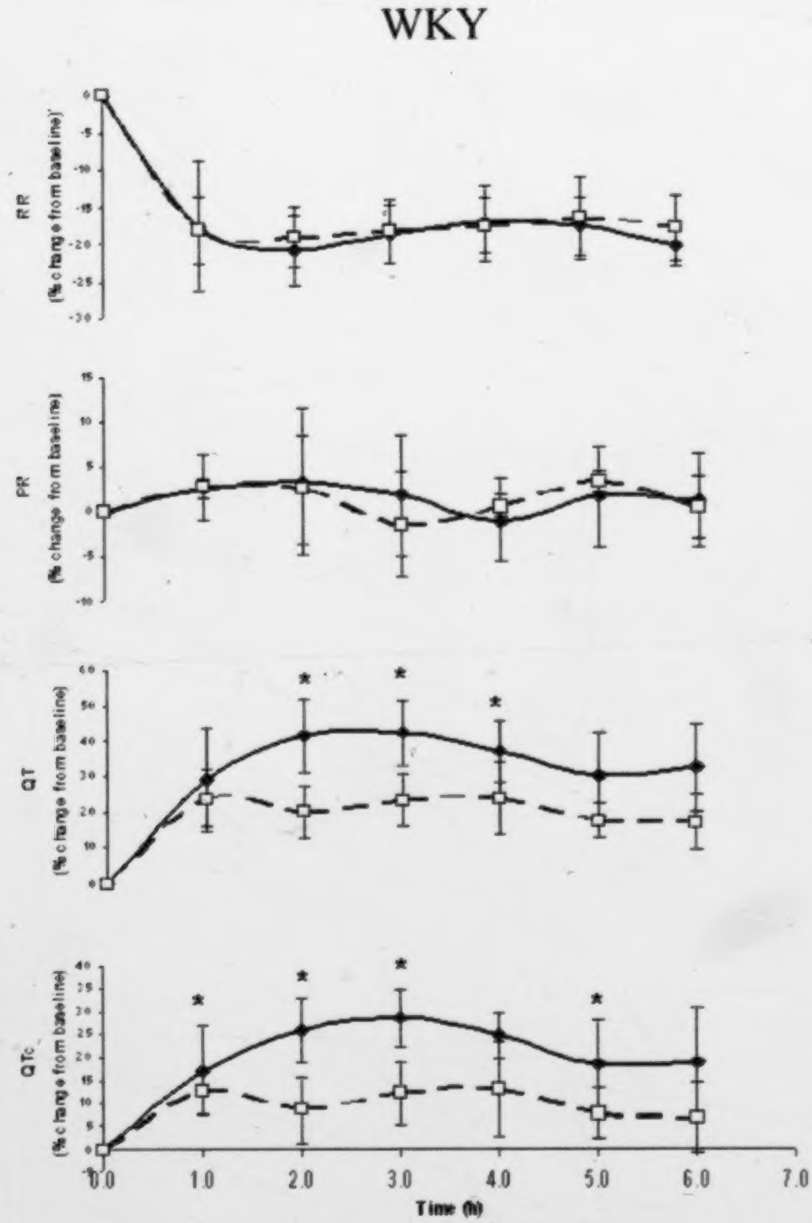


Figure 4-3: Mean ± SD of the time courses of sotalol % effect from baseline following administration of single oral doses of 80 mg/kg sotalol racemate to control (◆) and $IFN_{\alpha 2a}$ (□) treated rats. * Significant difference between control and $IFN_{\alpha 2a}$ (Student's t-test for unequal variance). Control (n=6) and Treated (n=6).

SHR

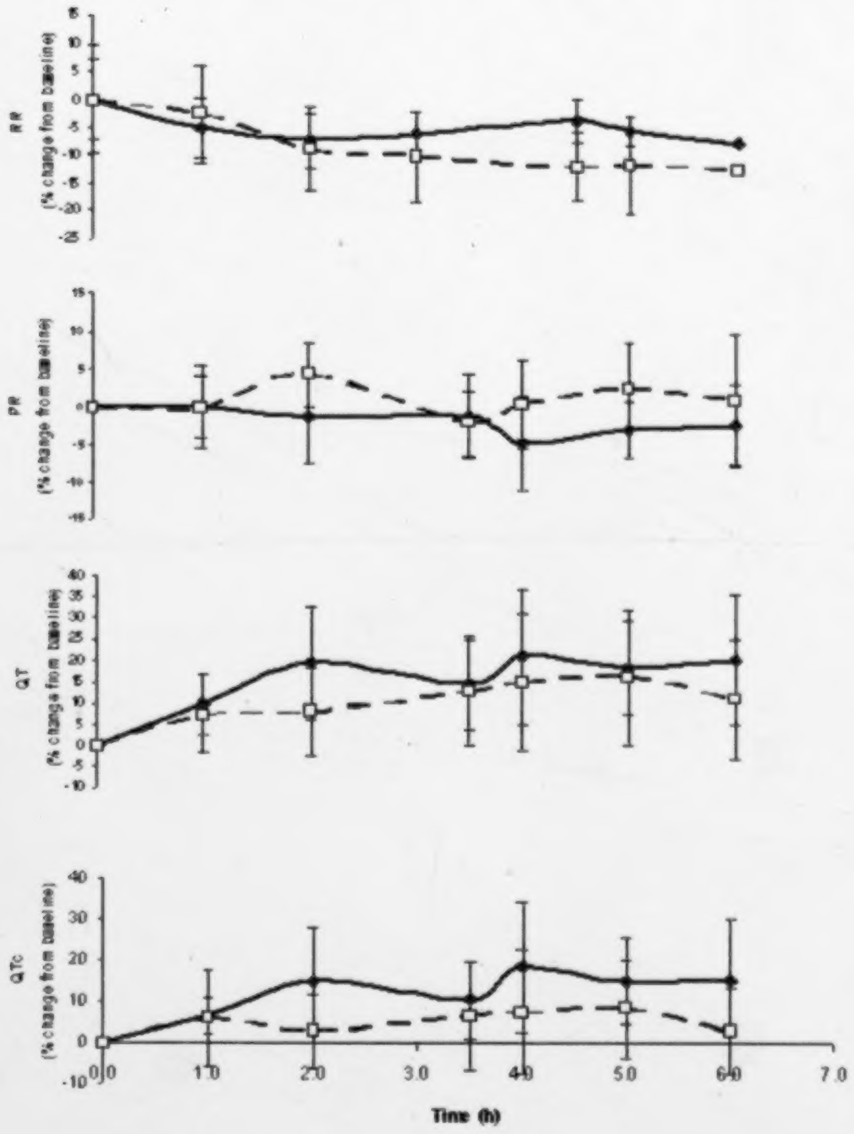


Figure 4-4: Mean ± SD of the time courses of sotalol % effect from baseline following administration of single oral doses of 80 mg/kg sotalol racemate to control (◆) and IFN$_{\alpha 2a}$ (□) treated rats. No significant effect was observed in any group (Student's t-test for unequal variance). Control (n=5) and Treated (n=6).

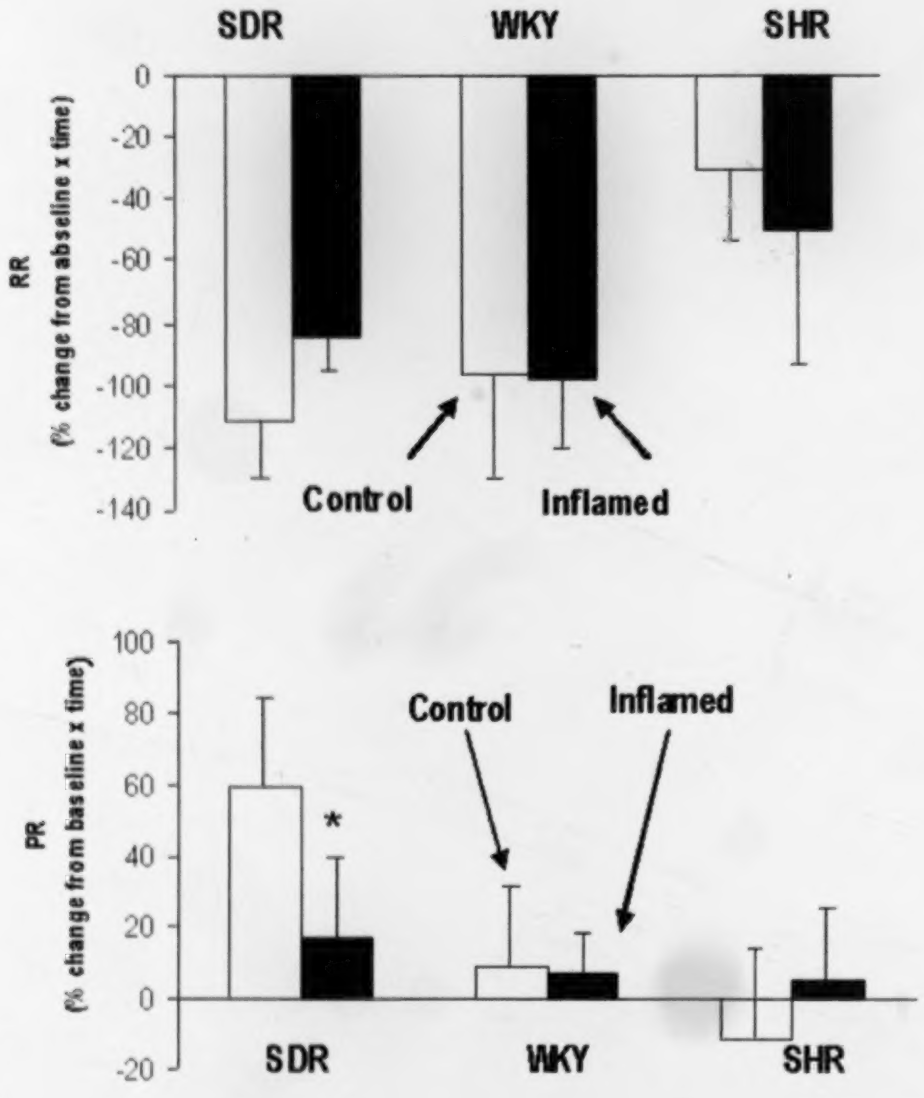


Figure 4-5: Mean ± SD of the response to sotalol in control (open bars) versus $IFN_{\alpha 2a}$ (close bars) treatment between strains. AUEC $_{0\text{-}6h}$ was depicted for heart rate (RR) and PR intervals. Error bars represent standard deviation of the mean. * Significantly different from respective Control (Student's t-test for unequal variance). SDR (n=4/group), WKY (n= 6/group), SHR (n=5-6/group).

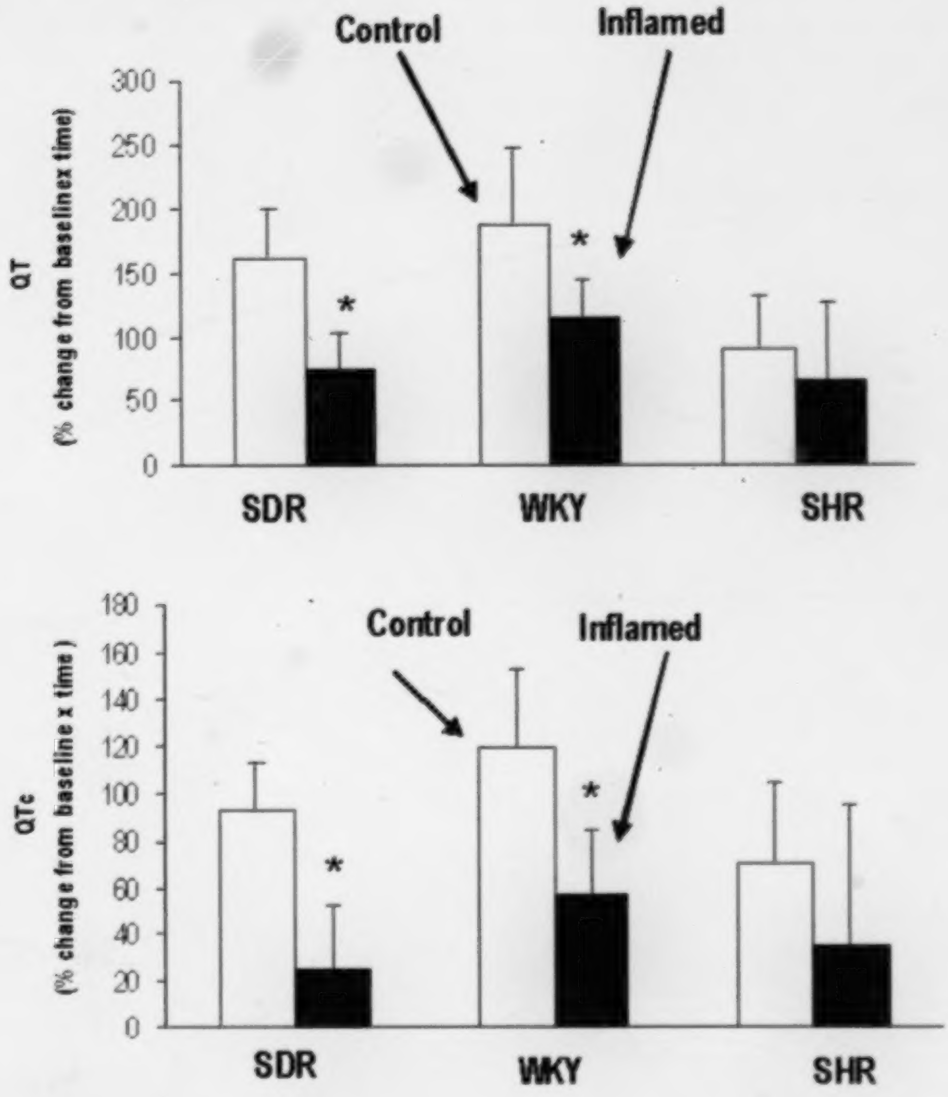


Figure 4-6: Mean ± SD of the response to sotalol in control (open bars) versus IFN$_{\alpha 2a}$ (close bars) treatment between strains. AUEC $_{0\text{-}6h}$ was depicted for QT and QTc intervals. Error bars represent standard deviation of the mean. * Significantly different from respective Control (Student's t-test for unequal variance). SDR (n=4/group), WKY (n=6/group), SHR (n=5-6/group).

Table 4-4: Mean ± SD of AUEC $_{0\text{-}6h}$ for heart rate (RR), QT, PR and QTc intervals after sotalol treatment.

AUEC$_{0\text{-}6h}$ Sotalol Effect (Area under the % change in effect from baseline x time)			
	Control (saline)	**Inflamed (IFN $_{\alpha 2a}$)**	**P value**
RR			
SDR (n=4/group)	-110.8 ± 19.2	-84.2 ± 11.1	0.062
WKY (n= 6/group)	-96.7 ± 33.3	-98.2 ± 22.0	0.93
SHR (n=5-6/group)	-30.6 ± 22.9 [*]	-50.7 ± 42.6	0.34
P value strain effect	**0.0007**	0.06	
PR			
SDR (n=4/group)	59.3 ± 24.8 [*]	16.8 ± 23.1 [#]	**0.046**
WKY (n= 6/group)	9.2 ± 22.3	7.6 ± 10.5	0.89
SHR (n=5-6/group)	-11.0 ± 24.9	5.8 ± 19.6	0.22
P value strain effect	**0.002**	0.64	
QT			
SDR (n=4/group)	162.8 ± 37.0	74.1 ± 29.6 [#]	**0.01**
WKY (n= 6/group)	187.2 ± 59.6	116.5 ± 27.1 [#]	**0.0035**
SHR (n=5-6/group)	89.8 ± 42.0 [*]	65.1 ± 62.7	0.44
P value strain effect	**0.009**	0.2	
QTc			
SDR (n=4/group)	92.8 ± 20.9	24.9 ± 27.9 [#]	**0.0055**
WKY (n= 6/group)	118.9 ± 33.5	56.8 ± 27.2 [#]	**0.01**
SHR (n=5-6/group)	70.6 ± 33.7	34.0 ± 60.9	0.23
P value strain effect	0.06	0.53	

[*] Significant difference between strains (one way ANOVA followed by the Duncan's New Multiple Range test at $\alpha = 0.05$).

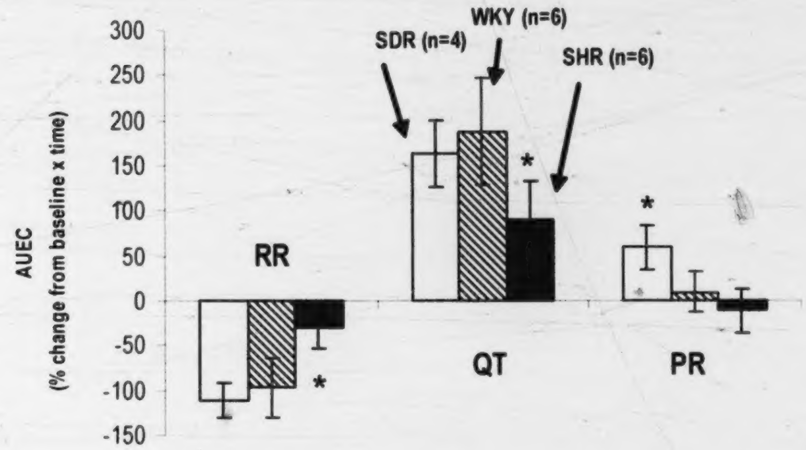
[#] Significantly different from respective Control (Student's t-test for unequal variance).

Table 4-5: Mean ± SEM for baseline difference in ECG parameters among rat strains.

	SDR (n=8)	WKY (n= 12)	SHR (n=11)	P value
RR (BPM)	381.5 ± 6.12	359.0 ± 5.75	326.8 ± 5.95*	0.0000057
PR (msec)	47.1 ± 1.53	48.7 ± 0.39	49.0 ± 0.78	0.32
QT (msec)	69.0 ± 0.85	71.3 ± 1.03	71.7 ± 0.78	0.11
QTc (msec)	173.7 ± 1.7	174.0 ± 2.0	167.8 ± 2.6	0.08

* Significantly different from other groups (one way ANOVA followed by the Duncan's New Multiple Range test at $\alpha = 0.05$).

Strain Difference in Sotalol Response in Control Animals



Strain Difference in Sotalol Response In Inflamed Animals

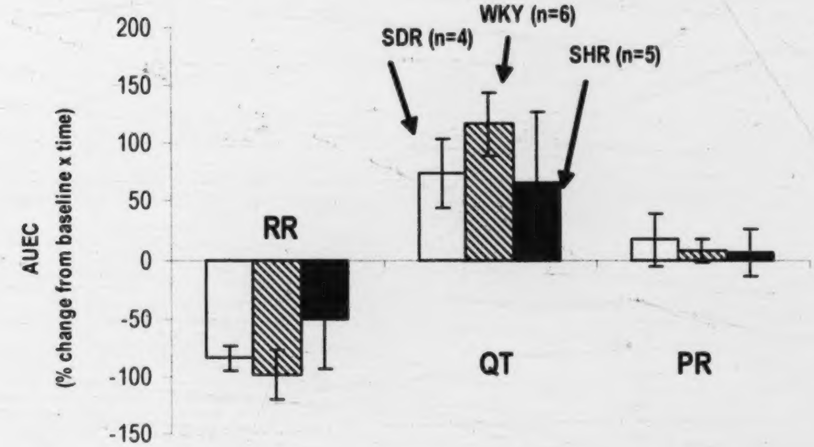


Figure 4-7: Mean ± SD of the response to sotalol, compared between rat strains in control (top graph) and IFN$_{\alpha 2a}$ (bottom graph). AUEC $_{0\text{-}6h}$ was depicted for heart rate (RR), QT and PR intervals. * Significantly different between strains (one way ANOVA followed by the Duncan's New Multiple Range test at $\alpha = 0.05$). SDR (n=4/group), WKY (n=6/group), SHR (n=5-6/group).

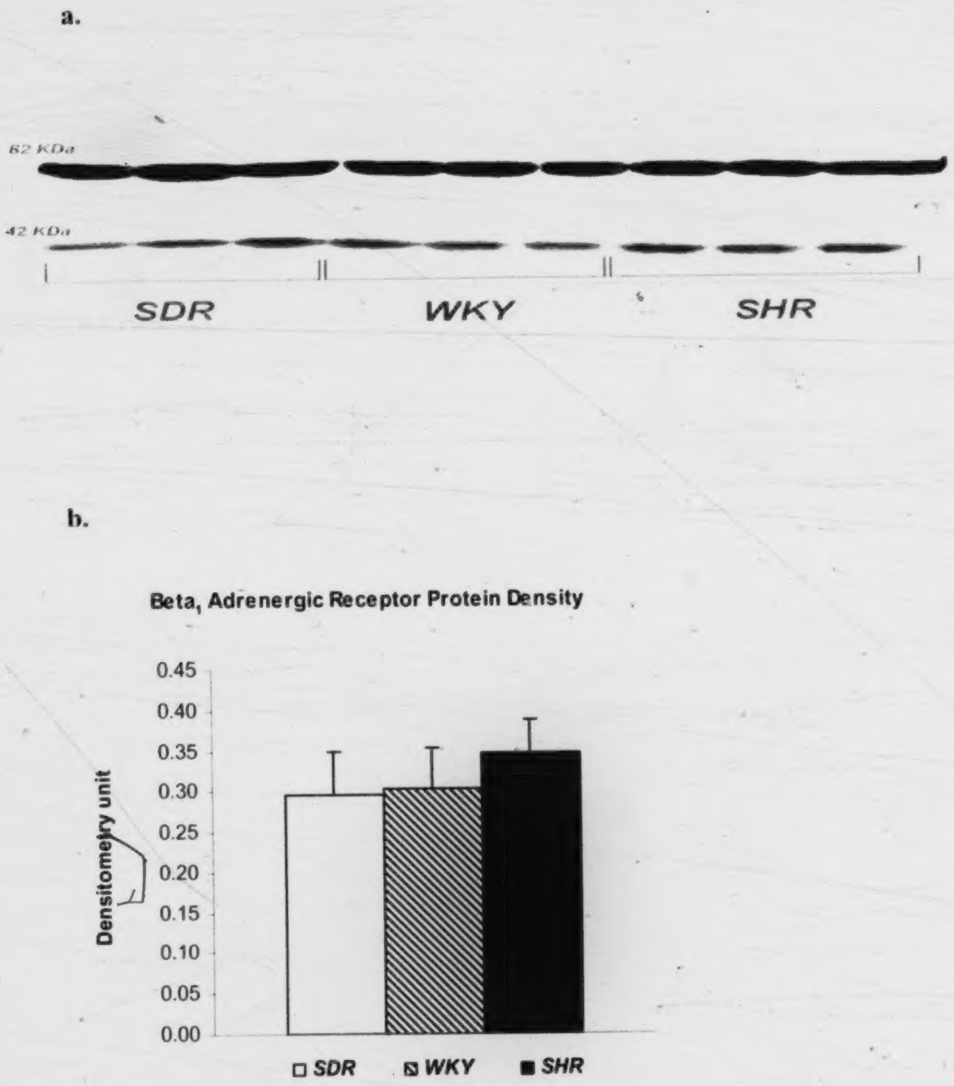


Figure 4-8: Western blot analysis of β_1AR protein expression in the whole heart (n=3/strain) (a) The β_1AR protein expression is 62 kDa and the internal standard β-actin is present at 42 KDa. Densitometric analysis of the β_1AR protein (b). No significant difference between groups.

Discussion:

Previous studies have shown significantly reduced potency of β-adrenergic receptor, calcium channel and potassium channel antagonists in presence of inflammation Kulmatycki et al, 2001; Guirguis & Jamali 2003). Both acute inflammation caused by IFN$_{\alpha 2a}$ and chronic inflammation (adjuvant arthritis induced) reduced sotalol (Kulmatycki et al 2001) and propranolol (Guirguis & Jamali 2003) potency in the rat. This decrease in beta-blocker effectiveness appeared to be independent of pharmacokinetic alterations and more possibly due to pharmacodynamic changes. Increasing evidence suggest there are changes to the actual receptor effectiveness, which can be attributed to increasing levels of the inflammatory mediators. Receptor responsiveness may be decreased due either to a decrease in expression, altered receptor activity, or changes to the intracellular signaling. (Bernardin et al., 1998; Hammond et al., 1992; Kompa et al., 1999; Krause et al., 1995; Kadoi et al., 2002). The observed changes in pharmacodynamics of these cardiovascular drugs in inflammation would therefore have important therapeutic consequences. Patients who have underlying auto-immune disease, such as RA, are at a higher risk of developing cardiovascular complications (Biasucci et al., 1999; McEntegart et al., 2001; Wallberg-Jonsson et al., 2000). Indeed, the primary cause of mortality in RA patients is due to cardiovascular complications (Prior et al., 1984; Mutru et al., 1985; Myllykangas-Luosujarvi et al., 1995; Wolfe et al., 1994). Reduced receptor responsiveness to drug therapy is a plausible reason for therapeutic failure of the cardiovascular drugs in patients who have hyper-inflammatory conditions who also have cardiovascular diseases. Indeed, a series of studies implicate increasing pro-inflammatory mediators such as TNFα, interleukin 6, and monocytes in cardiac diseases (Dorffel et al., 1999; Koller-Strametz et al., 1998; Liu et al., 1999). It appears that the proinflammatory mediators may be involved directly in decreasing β-adrenergic receptor responsiveness to drug treatment. Sattari et al (2003) have implicated increasing pro-inflammatory levels with decreased expression of β_1AR of inflamed normotensive rats. It may be that the existing hyper-inflammatory state, prevalent in the cardiovascular diseases, is responsible for the observed reduced drug potency. However, animal models presently used to demonstrate reduced responsiveness to cardiovascular drugs in inflammatory diseases have been normotensive. A more realistic animal model that would reflect human patho-physiological disease states would further provide relevant information with regards to the underlying cause of drug inefficacy in inflammatory states. We, therefore, decided to test the Spontaneously Hypertensive rat (SHR) model and its normotensive counterpart Wistar Kyoto (WKY) rat model as possible models for further elucidation of the mechanisms of β-adrenergic receptor down-regulation as compared to the already established Sprague-Dawley rat (SDR) inflammatory model. SHR and WKY models were chosen for study due to their extensive use as models for human cardiovascular studies (Handbook Of Lab Animal Science., 1994).

The present data suggest that there is a strain difference with regards to baseline inflammatory state, as well as response to the cardiovascular drug sotalol in both control and $IFN_{\alpha 2a}$ treated groups.

Sprague-Dawley rats were introduced into the study as a positive control. Treatment of SDR strain with the cardiovascular drug sotalol prolonged the PR and QT intervals as expected. Sotalol is a racemic drug, in which R-sotalol is a beta adrenergic receptor antagonist and S-sotalol is a specific potassium channel blocker (Anderson et al., 1999). It blocks the potassium channel by binding to the active state of the ATP-sensitive delayed inward rectifier potassium channel (Ik_1), or the ether-a- go-go (HERG) channel, found in both the human and rat hearts (Numaguchi et al., 2000; Pond et al., 2001). Induction of inflammation by $IFN_{\alpha 2a}$ caused a significant reduction in the ability of sotalol to prolong PR and QT intervals in the SDR strain as had been noted previously (Table 4-4, Figure 4-2). In all our strains, the increase in the inflammatory state was observable with the significantly higher levels of segmented neutrophil levels as compared to baseline (Table 4-1). Studies have positively correlated increasing inflammatory states and high inflammatory mediator with increase in the segmented neutrophil levels (Terashima et al., 2001). This suggests, in the absence in inflammatory mediator level data, segmented neutrophil counts maybe reliably used to reflect increasing inflammatory states.

Similar to its effect on the SDR strain, sotalol prolonged the QT and the QTc interval in the normotensive WKY strain (Table 4-3), and the sotalol potency on the QT and the QTc interval prolongation was reduced by inflammation in the WKY strain (Figure 4-3). It appears that varying forms of inflammatory diseases, such as myocardial infarction and cardiac heart failure, down-regulate the Ik_1 potassium channels possibly at the genetic level (Brooksby et al., 1994; Huang et al., 2000). Hence, the decreased QT interval prolongation in sotalol-treated SDR and WKY strains with inflammation is due, perhaps, to down-regulation in the Ik_1 potassium channel and/or HERG channel expression or activity in the rat heart with increasing inflammation.

Unlike the normotensive rat strains however, there was no difference between control and inflamed hypertensive SHR strains in the QT and QTc interval prolongation with sotalol dosing (Figure 4-6). Indeed, there appears to be no difference in sotalol efficacy between control and inflamed groups in any of the ECG parameters in the SHR strain (Figure 4-4, Table 4-4). The control group of the SHR strain also displayed a significantly lower sotalol potency compared to the SDR and WKY strains (Figure 4-7). One consideration for the lack of sotalol efficacy in the hypertensive rat strain is the possible changes to the pharmacokinetics of sotalol, a drug that is renally excreted, due to altered kidney function in the SHR strain. Evidence indicates that the possible pathogenesis of hypertension in the SHR strain may have its origin in the kidneys, and alterations to the renal function will potentially affect the pharmacokinetics of renally excreted drugs (Uber et al., 1996; Kopf et al., 1993). The renal function of the SHR strain has been shown to decrease with age in particular. Older SHR rats of 14 month old SHR rats appear to have increased glomerular albumin excretion, in comparison with age-matched WKY rats. Changes to the renal function were not evident in younger SHR rats of 3 or 9 month old in age (Bakoush et al., 2004). The SHR rats obtained for our study were 13 weeks of age, and therefore it is unlikely that renal failure had developed which was severe enough to have affected the pharmacokinetics of sotalol.

Another consideration is that the lack of PR interval prolongation is also observed in the normotensive WKY rats (Table 4-4, Figure 4-5). As the renal function of WKY is not known to be affected by age or genetic makeup, there should not have been a change in sotalol pharmacokinetics in the WKY rats. Yet difference in sotalol efficacy is observed in both SHR and WKY strains, as compared with the SDR strain, with regards to PR interval prolongation. A difference in renal function, and thus altered sotalol clearance, is therefore an insufficient and unlikely explanation for the differences observed among the strains.

A more likely explanation for altered sotalol efficacy lies with the possible altered receptor responsiveness to sotalol treatment in the WKY and SHR strains compared to the SDR strain. The QT interval prolongation with sotalol treatment involves the direct inhibition of the potassium, in particular the Ik_I, channel by sotalol. Lack of sotalol effect in QT interval prolongation with the hypertensive SHR strain (Table 4-4, Figure 4-6) may be due to differences in the Ik_I current. Studies have indicated SHR strains exhibit a lower Ik_I current density as compared to WKY strains, possibly leading to the prolonged action potential associated with hypertrophic hearts (Li et al., 2000). A lower Ik_I density may partially explain the absence of response seen in the hypertensive strain with sotalol treatment. The decrease in sotalol efficacy in SHR strain may also be related to the presence of a recently defined inhibitory protein partner capable of limiting the pharmacological sensitivity of HERG called KCR1 (Kupershmidt et al., 2003). Functionally, KCR1 reduces the sensitivity of HERG channel to classic pro-arrhythmic HERG blockers such as sotalol and quinidine in both cardiac and non-cardiac cell lines. The possibility of the SHR strain over expressing the KCR1 protein as compared the normotensive strains may explain the decrease in sotalol potency in control SHR strain.

PR interval prolongation by sotalol, as seen in the SDR strain (Table 4-4, Figure 4-5), involves both direct and indirect inhibition of calcium trafficking through β_1AR blockade (Putney Jr., 1993). It is one of the mechanisms by which sotalol is able to control heart rate and decrease cardiac output. In the WKY and SHR strains however, sotalol did not alter the PR interval in either the control or inflamed groups (Figure 4-7). Possible reasons for the observed divergence between the strains may be variation in protein expression of the β_1 adrenergic receptor of the SDR strain, as compared to the WKY and SHR strain, leading to altered sotalol potency. A western blot analysis of the β_1AR protein expression on the heart however indicated no significant difference in the protein levels (Figure 4-8).

Several explanations can be offered for the lack of sotalol response in PR interval prolongation in WKY and SHR strains. Studies have shown strain difference between SDR, WKY and SHR in response to β adrenergic modulation. One study investigated the response of β adrenoceptor modulation of pressure-dependent renin release with propranolol. While in the SDR strain, renin release was significantly reduced, similar to human models, propranolol appeared to have no response in WKY and SHR strains, and renin release was not altered (Porter., 1992). Aside from receptor modulation, downstream receptor activity may differ among strains, especially the Gs protein signaling mechanism. Although not studied in the rat model, altered Gs signaling have been implicated for reduced drug potency in other animal models (Feldman et al., 1990). Calcium handling may also be another parameter in which rat strains vary, leading to the altered sotalol potency among strains. β adrenergic responsiveness is partly determined by sarcoplasmic reticulum (SR) regulation of calcium release. Calcium release from the SR with βAR stimulation will, in turn, stimulate calcium entry from the L-type Ca^{2+} channels. Therefore three parameters, SR-related Ca^{2+} handling, Ca^{2+} channel expression and/or Ca^{2+} import may be varied among strains. Although SHR strain appears to have impaired β adrenergic response to stimulation with increasing hypertrophic state, sarcoplasmic reticulum-related Ca^{2+} handling appear not to be different between SHR and WKY strains (Keller et al., 1997). Studies have yet to indicate whether SDR strain varies in intracellular Ca^{2+} handing. Interestingly, radioligand binding studies with [+3H] Ca^{2+} antagonists failed to show a significant difference between the maximum binding (Bmax) obtained for the cardiac membranes of 9-week-old, or 25 week old SDR, WKY, or SHR strains (Dillon et al., 1989). However when Ca^{2+} import, a function of contraction in the aortic smooth muscle from the calcium channels, was studied on its own between the three strains, the SDR strain appeared to have more Ca^{2+} import as compared to WKY and SHR strains with external stimulation (Rahmani et al., 1999).

Studies often highlight differences in pathophysiology and response between SHR and WKY strains. However, one must use caution in these strains as appropriate models for the study of inflammatory-drug-disease interaction. The SHR strain develops the etiology of hypertension state early in age, at 5 weeks. At the later stages of its life, as the SHR strain gets old, hyper-inflammation, renal failure, and cardiac hypertrophy ensue, reflective of the human condition (Handbook of Lab Animal Science., 1994). Literature reports on the rat ECG values of the SHR strain, as well as drug response to PR and QT intervals, however, are highly inconsistent and variable (Baillard et al., 2000; Lu et al., 1999; Yamori et al., 1976). Varied methods of measurement, age of the rats used, and experimental protocols may explain part of the observed variability. Also, the use of WKY strain as the normotensive counterpart for SHR is coming into question, as they appear to be genetically ill defined and thus its validity for this purpose may be doubtful (Gill et al., 1985). Baseline PR and QT interval prolongation observed by the SDR strain, as well as the response observed with β adrenergic antagonists are, however, comparable to results seen in humans with sotalol treatment (Anderson et al., 1999).

This study has a few limitations. Although Ca^{2+} channel availability does not appear to be different between strains, Ca^{2+} channel protein expression has not yet been studied, and may be an important determinant of PR interval variations observed among strains. Also, the lack of proper pro-inflammatory levels, namely CRP, TNFα and NO, prevents a full interpretation of the results.

In conclusion, further studies would be required to elucidate the molecular mechanisms by which various rat strains respond to beta adrenergic and potassium channel antagonist treatment. Meanwhile, care must be taken in choosing an appropriate rat model for the study of electrocardiographic changes that occur with inflammatory-drug-disease interactions.

Chapter 5: General Conclusion

More and more research with regards to inflammation and disease states express a common theme, in which the interplay between increasing pro-inflammatory mediators and physiological manifestations is revealed. Increasing inflammatory states promote, exacerbate, and eventually lead to possible organ failure and death. There is also an increase in pro-inflammatory mediator levels, and a decrease in anti-inflammatory mediators, in cardiovascular diseases such as cardiac heart failure, ischemia, stroke, and renal failure. It now appears that the increase in the pro-inflammatory mediators have a role in altering how a drug can be absorbed, distributed, metabolized, and/or excreted, influencing the level of therapeutic efficacy that can be achieved with drug treatment. For example, cardiovascular drugs such as verapamil and propranolol have decreased clearance in inflammatory states, leading to an increase in concentration. Alterations that occur to drug levels are important to keep under consideration, as traditionally, increase in concentration is positively correlated to effect. More reports indicate, however, that although drug concentration may be significantly higher in inflammation, drug efficacy may be attenuated, as were seen in rheumatoid arthritis patients. For example, when verapamil was administered, it exhibited a significantly lower response despite the increase in concentration. Similar results were obtained in rat models of inflammation with beta adrenergic receptor antagonists. A significant correlation between altered drug pharmacokinetics and pharmacodynamics and inflammatory mediator levels hint at their possible role in the decrease in response. Reduced potency of these first line antihypertensive drugs in inflammatory states would bring into concern the therapeutic efficacy of other cardiovascular drugs administered with hyper-inflammatory states, and their therapeutic relevance. It appears that the effect of inflammatory mediators on the beta adrenergic and calcium channel antagonists cannot be generalized to all cardiovascular drugs. Our present clinical study with the angiotensin receptor antagonists' valsartan and losartan suggests that inflammation, at least, as manifested by rheumatoid arthritis, does not decrease the efficacy of angiotensin receptor antagonist. In fact, the possibility of increased response with valsartan, due to a possible angiotensin receptor up-regulation, is intriguing. Further studies must be done regarding their therapeutic use to treat cardiovascular conditions in the setting of inflammation, over calcium channel blockers or beta adrenergic antagonists. As well, it is important to study pharmacokinetic and pharmacodynamic alterations of cardiovascular drugs with inflammation when there is an underlying hypertensive state present. To establish such a cardio-inflammatory disease model, we decided to first ascertain whether existing hypertensive animal models are feasible models to use for the human cardio-inflammatory conditions. The normotensive Sprague Dawley rat had already proved to be a useful animal model for the human condition to study the pharmacokinetics and pharmacodynamic alterations of beta adrenergic receptor antagonists such as sotalol in inflammatory states. We therefore compared the effect of sotalol in the spontaneously hypertensive rat strain and the normotensive Wistar Kyoto strain in the control and inflamed groups to that of the Sprague Dawley rat strain. Our preliminary study shows that due to differences not related to the hypertensive state of the strains, the spontaneously hypertensive rat strains would not be a good model to study the down-regulation of cardiovascular drugs seen in inflammation. The effect of sotalol seen in the two test strains does not reflect what is observed with humans. Thus, care must be exercised when interpreting results obtained using different rat strains as cardio-inflammatory models.

All in all, a clear understanding of the down-regulation phenomenon observed with cardiovascular drugs would be beneficial in optimal patient treatment. The future of drug research and disease-drug interaction would lie in determining the mechanisms by which inflammation-related alteration in receptor responsiveness is being mediated.

References:

Abdel-Razzak Z, Loyer P, Fautrel A, Gautier J-C, Corcos L, Turlin B, Beaune P, Guillouzo A. Cytokines down-regulate expression of major cytochrome P-450 enzymes in adult human hepatocytes in primary culture. *Mol Pharmacol* 1993; 44: 707-715.

Achira M, Totsuka R, Fujumura H, Kume T. Tissue-specific regulation of expression and activity of P-glycoprotein in adjuvant arthritis rats. *European J Pharm Sci* 2002; 16:29-36.

Adamson PB, Vanoli E, Hull SS, Foreman RD, Schwartz PJ. Antifibrillatory efficacy of ersentilide, a novel beta-adrenergic and Ikr blocker, in conscious dogs with a healed myocardial infarction. *Cardiovasc Res* 1998; 40: 56-63.

Advani SV, Singh BN. Pharmacodynamic, pharmacokinetic and antiarrhythmic properties of d-sotalol, the dectro-isomer of sotalol. *Drugs* 1995; 49: 664-679.

Akishita M, Horiuchi M, Yamada H, Zhang L, Shirakami G, Tamura K, Ouchi Y, Dzau VJ. Inflammation influences vascular remodeling through AT2 receptor expression and signaling. *Physiol Genomics* 2000 ; 2:13-20

Albert MA, Danielson E, Rifai N, Ridker PM for the PRINCE investigators. Effect of statin therapy on C-reactive protein levels. *JAMA* 2001; 286: 64-74.

Álvarez Á, Hermenegildo C, Issekutz AC, Esplugues JV, Sanz M-J. Estrogens inhibit angiotensin II-induced leukocyte-endothelial cell interactions in vivo via raid endothelial nitric oxide synthase and cyclooxygenase activation. *Circ Res* 2002; 91: 1142-1150.

Anderson JL, Prystowsky E. Sotalol: an important new antiarrhythmic. *Am Heart J* 1999; 137: 388-409.

Animal Models: Assessing the Scope of Their Use in Biomedical Research. Progress in Clinical and Biological Research Vol 229. Written by Walter Lovenberg . edited by Junichi Kawamata and Edward C. Melby, Jr. 1985. p. 225-240.

Ansher S, Thompson W, Snoy P, Habig W. Role of endotoxin in alterations of hepatic drug metabolism by diphtheria and tetanus toxoids and pertussis vaccine adsorbed. *Infect Immun* 1992; 60: 3790-3798.

Antonelli G, Currenti M, Turrizianin O, Dianzani F. Neutralizing antibodies to interferon-alpha: relative frequency in patients treated with different interferon preparations. *J Infect Dis* 1991; 163: 882-885.

Archer S, Shultz P, Warren J, Hampl V, Demaster E. Preparation of standards and measurement of nitric oxide, nitroxyl and related oxidation products. *Methods Enzymology* 1995; 7:21-24.

Arici M, Walls J. End-stage renal disease, atherosclerosis, and cardiovascular mortality: is C-reactive protein the missing link? *Kidney Int* 2001; 59: 407-414.

Asselbergs FW, van Boven AJ, Stuveling EM, Diercks, GFH, Hillege HL, Kors JA, de Jong PE, van Gilst WH, for the PREVEND study group. Relation of electrocardiographic abnormalities to levels of serum C-reactive protein. *Am J Cardiol.* 2003 ; 91:1358-1360.

Ayrton A, Morgan P. Role of transport proteins in drug absorption, distribution and excretion. *Xenobiotica* 2001; 31: 469-497.

Bakoush O, Tencer J, Torffvit O, Tenstad O, Skogvall I, Rippe B. Increased glomerular albumin permeability in old spontaneously hypertensive rats. *Nephrl Dial Transplant* 2004; 19: 1724-1731.

Balbay Y, Tikiz H, Baptiste RJ, Ayaz S, Sasmaz H, Korkmaz S. Circulating interleukin-1 beta, interleukin-6, tumor necrosis factor-alpha, and soluble ICAM-1 in patients with chronic stable angina and myocardial infarction. *Angiology* 2001; 52:109-114.

Bachetti T, Comini L, Pasini E, Cargnoni A, Curello S, Ferrari R. Ace-inhibition with quinapril modulates the nitric oxide pathway in normotensive rats. *J Mol Cell Cardiol* 2001; 33: 395-403

Bacon CM, Petricoin EF 3rd, Ortaldo JR, Rees RC, Larner AC, Johnston JA, O'Shea JJ. Interleukin 12 induces tyrosine phosphorylation an activation of STAT4 in human lymphocytes. *Proc Natl Acad Sci* USA 1995; 92 730-7311.

Baillard C, Mansier P, Ennezat PV, Mangin L, Medigue C, Swyndhedauw B, Chevalier B. Converting enzyme inhibition normalizes QT interval in spontaneously hypertensive rats. *Hypertension* 2000; 36: 350-354.

Barki-Harrington L, Luttrell LM, Rockman HA. Dual inhibition of beta-adrenergic and angiotensin II receptors by a single antagonist: a functional role for receptor-receptor interaction in vivo. *Circulation*. 2003; 108:1611-1618.

Biasucci LM, Liuzzo G, Fantuzzi G, Caligiuri G, Rebuzzi AG, Ginnetti F, Dinarello CA, and Maseri A. Increasing levels of interleukin (IL) -1Ra and IL-6 during the first 2 days of hospitalization in unstable angina are associated with increased risk of in-hospital coronary events. *Circulation* 1999; 99: 2079-2084.

Beierwaltes WH, Arendshorst WJ, Klemmer PJ. Electrolyte and water balance in young spontaneously hypertensive rats. *Hypertension*. 1982; 4: 908-915.

Belpaire FM, Bogaert MG, Rosseneu M. Binding of beta-adrenoceptor blocking drugs to human serum albumin, to alpha 1-acid glycoprotein and to human serum. *Eur J Clin Pharmacol.* 1982; 22: 253-256.

Benet LZ, Wu CY, Hebert MF, Wacher VJ. Intestinal drug metabolism and antitransport processes: a potential paradigm shift in oral drug delivery. *Journal of Controlled Release* 1996; 39: 139-144.

Berk BC, Corson MA. Angiotensin II signal transduction in vascular smooth muscle: role of tyrosine kinases. *Circ Res* 1997; 80: 607-616.

Bernardin G, Strosberg AD, Bernard A, Mattei M, Marullo S. β-adrenergic receptor-depended and independent stimulation of adenylate cyclase is impaired during severe sepsis in humans. *Intensive Care Med* 1998; 24: 1315-1322.

Bernton EW, Beach JE, Holaday JW, Smallridge RC and Fein HG. Release of multiple hormones by a direct action of interleukin 1 on pituitary cells *Science* 1987; 238: 519-521.

Bertilsson PM, Olsso P, Magnusson K-R. Cytokines Influence mRNA Expression of Cytochrome P450 3A4 and MDRI in Intestinal Cells. *J Pharm Sci* 2001; 90: 638-646.

Betz M, Fox BS. Regulation and development of cytochrome-c specific IL-4 producing T cells. *J Immunol* 1990; 145: 1046-1052.

Bianchi G, Baer PG, Fox U, Duzzi L, Pagetti D, Giovannetti AM. Changes in renin, water balance, and sodium balance during development of high blood pressure in genetically hypertensive rats. *Circ Res* 1975; 36: 153-161.

Biffl WL, Moore EE, Moore FA, Barnett CC, Silliman CC, Peterson VM. Interleukin 6 stimulates neutrophil production of platelet activating factor. *J Leuk Biol* 1996; 59: 569-574.

Biosource International, Human and Rat TNFα Immunoassay Kit Camarillo, California 93012 USA. Product datasheet and FAQs from: www.biosource.com/content/catalogContent/detail2.asp?partnum=LHC3011

Black RA, Rauch CT, Kozlosky CJ, Peschon JJ, Slack JL, Wolfson MF, Castner BJ, Stocking KL, Reddy P, Srinivasan S, Nelson N, Boiani N, Schooley KA, Gerhart M, Davis R, Fitzner JN, Johnson RS, Paxton RJ, March CJ, Cerretti DP. A metalloproteinase disintegrin that releases tumor-necrosis factor α from cells. *Nature 1*997; 385: 729-733.

Bocci V. What roles have anti-interferon antibodies in physiology and pathology? *Rec Clin Lab* 1991; 21. 79-84.

Borgstrom S, Von Eyben FE, Flodgren P, Axelsson B, Sjogren HO. Human leukocyte interferon and cimetidine for metastatic melanoma. *N Engl J Med* 1982; 307: 1080-1081.

Borish L, Posenbaum R, Albury L, Clark S. Activation of neutrophils by recombinant interleukin-6. *Cell Immunol* 1989; 121: 280-289.

Bradford MM. A rapid and sensitive method for the quantitation of microgram of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976; 72: 248-254.

Brasier AR, Recinos III A, Eledrisi MS. Vascular inflammation and the renin-angiotensin system. *Arterioscler Thromb Vasc* Biol 2002; 22: 1257-1266.

Brenner BM, Cooper ME, Zeeuw D, Keane WF, Mitch WE, Parving HH, Remuzzi G, Snapinn ST, Zhange Z, Shahinfar S. Effects of losartan on renal an cardiovascular outcomes in patients with type 2 diabetes and nephrophathy. *New Eng J Med* 2001; 345: 861-869.

Breitwieser GE. G protein-coupled receptor Oligomerization. Implications for G protein activation and cell signaling. *Circ Res.* 2004; 94: 17-27.

Brookman LJ, Rolan PE, Benjamin IS, Palmer KR, Wyld PJ, Lloyd P, Flesch G, Waldmierier F, Sioufi A, Mullins F. Pharmacokinetics of valsartan in patients with liver disease. *Clin Pharmacol Ther* 1997; 62: 272-278.

Brooksby P, Levi AJ, Jones JV. The electrophysiological characteristics of hypertrophied ventricular myocytes from the spontaneously hypertensive rat. *J Hypertens.* 1993; 11: 611-622.

Brugada P, Smeets JL, Brugada J, Farre J. Mechanism of action of sotalol in supraventricular arrhythmias. *Cardiovasc Drug Ther.* 1990; 3: 619-623.

Burnier M. Angiotensin II type 1 receptor blockers. *Circulation* 2001; 103: 904-912.

Butler DM, Maini RN, Feldman M, Brennan FM: Modulation of proinflammaotyr cytokine release in rheumatoid synovial membrane cell cultures: comparison of monoclonal anti TNF α antibody with interleukin 1 recpeor antagonist. *Eur Cytokine Netw* 1995; 6 : 225-230.

Campion GV, Lebsack ME, Lookabaugh J, Gordon G, Catalano M, IL-1 Ra Arthritis Study Group: Dose-range and dose-frequency study of recombinant human interleukin-1 receptor antagonist I patients with rheumatoid arthritis. *Arthritis Rheum* 1996; 39: 1092-1101.

Carlstedt F, Lind L, Lindahl B. Proinflammatory cytokines, measured in a mixed population on arrival in the emergency department, are related to mortality and severity of disease. *J Intern Med* 1997; 242:361-365.

Carey RM, Howell NL, Jin X-H, Siragy HM. Angiotensin Type 2 Receptor-Mediated Hypotension in Angiotensin Type-1 Receptor-Blocked Rats. *Hypertension* 2001; 38: 1272-1277.

Carrao S, Salli L, Arnone S, Scaglione R, Amato V, Cecala m, Licata A, Licata G. Cardiac involvement in rheumatoid arthritis: evidence of silent heart disease. *Eur Heart J* 1995; 16: 253-256

Carrao S, Salli L, Arnone S, Scaglione R, Pinto A, Licata A. Eco-doppler left ventricular filling abnormalities in patients with rheumatoid arthritis without clinically evident cardiovascular disease *Eur J Clin Invest* 1996; 26: 293-297.

Chin JE, Winterrowd GE, Krzesicki RF, Sanders ME . Role of cytokines in inflammatory synovitis: the coordinate regulation of intercellular adhesion molecule 1 and HLA class I and class II antigens in rheumatoid synovial fibroblasts. *Arthritis Rheum* 1990; 33: 1776-1786.

Choy EHS, Panayi GS. Cytokine pathways and Joint inflammation in rheumatoid arthritis. *N Engl J Med* 2001; 344: 907-916.

Christ DD. Human plasma protein binding of the angiotensin II receptor antagonist losartan potassium (Dup 753/MK 954) and its pharmacologically active metabolite EXP 3174. *J Clin Pharmacol* 1995; 35: 515-520.

Chen J-Q, Ström A, Gustafsson JA, Morgan ET. Suppression of the constitutive expression of cytochrome P-450 2C11 by cytokines and interferons in primary cultures of rat hepatocytes: comparison with induction of acute-phase genes and demonstration that *CYP2C11* promoter sequences are involved in the suppressive response to interleukins 1 and 6. *Mol Pharmacol* 1995; 47: 940-947.

Choi HK, Hernan MA, Seeger JD, Robins JM, Wolfe F. Methotrexate and mortality in patients with rheumatoid arthritis: a prospective study. *Lancet* 2002; 359: 1173-1177.

Chung MK, Martin DO, Sprecher D, Wazni O, Kanderian A, Carnes CA, Bauer JA, Tchou PJ, Niebauer MJ, Natale A, Van Wagoner DR. C-reactive protein elevation in patients with atrial arrhythmias. *Circulation* 2001; 104: 2886-2891.

Cindas A, Gökçe-Kutsal Y, Tokogözoglu L, Karanfil A. QT dispersion and cardiac involvement in patients with rheumatoid arthritis. *Scand J Rheumatol* 2002; 31: 22-26.

Clark WH. Tumour progression and the nature of cancer. *Br J Cancer* 1991; 64: 631-644.

Clarkson B, Strife A. Linkage of proliferative and maturational abnormalities in chronic myelogenous leukemia and relevance to treatment. *Leukemia.* 1993; 7: 1683-1721.

Cohn JN, Tognoni G. For the valsartan heart failure trial investigators: A randomized trial of the angiotensin-receptor blocker valsartan in chronic heart failure. *New Engl J Med* 2001; 345: 1667-1675.

Coleman JW. Nitric oxide: a regulator of mast cell activation and mast cell-mediated inflammation. *Clin Exp Immunol* 2002; 129: 4-10.

Colotta R, Re F, Muzio M, Polentarutti N, Minty A, Caput D, Ferrara P, Mantovani A.: Interleukin 1 type II receptor: a decoy target for IL-1 that is regulated by IL-4. *Science* 1993; 261: 472-475.

Cuzzocrea S, DeSarro G, Costantino G, Ciliberto G, Mazzon E, Se Sarro A, Caputti AP. IL-6 knockout mice exhibit resistance to splanchnic artery occlusion shock. *J Leuk Biol* 1999a ; 66: 471-480.

Cuzzocrea S, Sautebin L, De Sarro G, Constantino G, Rombola L, Mazzon E, Ialenti A, De Sarro A, Ciliberto G, Di rosa M, Caputti AP, thiemermann C. Role of IL-6 in the pleurisy and lung injury caused by carrageenan. *J Immunol.* 1999b; 163: 5094-5104.

Dahlöf B, Zanchetti A, Diez J, Nicholls MG, Yu CM, Barrios V, Aurup P, Smith RD, Johansson M; For the REGAAL Study Investigators. Effects of losartan and atenolol on left ventricular mass and neurohormonal profile in patients with essential hypertension and left ventricular hypertrophy. *J Hypertens* 2002; 20: 1879-1886

Dahlgren C, Karlsson A. Respiratory burst in human neutrophils. *J Immunol Methods.* 1999 ; 232: 3-14.

Dahl LK, Heine M, Tassinari L. Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt injection. *Nature* 1962; 431-456.

Dandona P, Kumar V, Aljada A, Ghanim H, Syed T, Hofmayer D, Moamty P, Tripathy D, Garg R. Angiotensin II receptor blocker valsartan suppresses reactive oxygen species generation in leukocytes, nuclear factor-kappa B, in mononuclear cells of normal subjects: evidence of an anti-inflammatory action. *J Clin Endocrinol Metab* 2003; 88: 4496-4501.

D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J Exp Med* 1993; 178: 1041-1048.

Daneshtalab N, Lewanczuk RZ, Jamali F. High-performance liquid chromatographic analysis of antiotensin II receptor antagonist valsartan using a liquid extraction method. *J Chromatogr B Analyt Technol Biomed Life Sci* 2002; 766: 345-349.

Da Silva JA, and Spector TD. The role of pregnancy in the course and aetiology of rheumatoid arthritis. *Clinical Rheumatology* 1992; 11: 189-194.

de Beer FC, Soutar AK, Baltz ML, Trayner I, Feinstein A, Pepys MB. Low density and very low density lipoproteins are selectively bound by aggregated C-reactive protein. *J Exp Med* 1982; 156: 230-242.

de Boer RA, Pinto YM, Suurmeijer AJ, Pokharel S, Scholtens E, Humler M, Saavedra JM, Boomsma F, van Gilst WH, van Veldhuisen DJ. Increased expression of cardiac angiotensin II type 1 (AT($_1$)) receptors decreases myocardial microvessel density after experimental myocardial infarction. *Cardiovasc Res* 2003; 57:434-442.

Dedhia HV, DiBartolomeo A. Rheumatoid Arthritis. *Critical Care Clinics* 2002; 18: 841-854.

Delaporte E, Renton KW. Cytochrome P450 1A1 and cytochrome P450 1A2 are downregulated at both transcriptional and post-transcriptional levels by conditions resulting in interferon-alpha/beta induction. *Life Sci* 1997; 60: 787-796.

del Rincón I, Williams K, Stern MP, Freeman GL, Escalante A. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis Rheum* 2001; 44: 2727-2745.

De Maeyer D. and De Maeyer-Guignard J. (1988) Interferons and Other Regulatory Cytokines (Wiley, New York), pp. 380-424.

Deswal A, Petersen NJ, Feldman AM, Young JB, White BG, Mann DL. Cytokines and cytokine receptors in advanced heart failure. An analysis of the cytokine ddatabase from the vesnarinone trial (VEST). *Circulation* 2001; 103: 2055-2059.

Deten A, Volz HC, Briest W, Zimmer HG. Cardiac cytokine expression is upregulated in the acute phase after myocardial infarction. Experimental studies in rats. *Cardiovasc Res* 2002; 55: 329-340.

Dillon JS, Gu XH, Nayler WG. Effect of age and of hypertrophy on cardiac Ca^{2+} antagonist binding sites. *J Cardiovasc Pharmacol* 1989;14 :233-240.

Di Napoli M, Pap F, Bocola V. C-reactive protein in ischemic stroke: an independent prognostic factor. *Stroke* 2001; 32: 917-924.

Dinarello CA. Proinflammatory cytokines. *CHEST* 2000; 118: 503-508.

Dorffel Y, Latsch C, Stuhlmuller B, Schreiber S, Scholze S, Burmester GR, Scholze J. Preactivated peripheral blood monocytes in patients with essential hypertension. *Hypertension* 1999; 34:113-117.

Dorian P, Newman D, Sheahan R, Tang A, Green M, Mitchell J. d-Sotalol decreases defibrillation energy requirements in humans: a novel indicaion for drug therapy. *J Cardiovascular Electrophysiol.* 1996; 7: 952-961.

Dostal DE, Hunt RA, Kule CE, Bhat GJ, Karoor BV, McWhinney DF, Baker KM. Molecular mechanisms of angiotensin II in modulating cardiac function: intracardiac effects and signal transduction pathways. *J Mol Cell Cardiol* 1997; 29: 2893-2902.

Dzimiri N. Receptor Cross-talk. Implications for cardiovascular function, disease and therapy. *Eur J Biochem* 2002; 269: 4713-4730.

Egger G, Sadjak A, Porta S, Purstner P, Gleispach H. Changes in blood catecholamines, insulin, corticosterone and glucose during the course of the Sephadex inflammation. *Exp Pathol* 1982; 21:215-219.

Egido J. Vasoactive hormones and renal sclerosis. *Kidney Int* 1996; 49: 578-597.

Elliott MJ, Maini RN, Feldman M, Kalden JR, Antoni C, Smolen JS, Leeb B, Breedveld FC, Macfarlane JD, Bijl H, Woody JN. Randomized double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor α (cA2) versus placebo in rheumatoid arthritis. *Lancet* 1994; 344: 1105-1110.

Feghali CA, Wright TM. Cytokines in acute and chronic inflammation. *Front Biosci* 1997; 2: d12-d26.

Feldman M, Elliott MJ, Woody JN, Maini RN. Anti-tumor necrosis factor-alpha therapy of rheumatoid arthritis. *Adv Immunol* 1997; 64: 283-350.

Feldman M, Maini R. Anti-TNF α therapy of rheumatoid arthritis: What have we learned? *Anu Rev Immunol* 2001; 19: 163-196.

Feldman AM, Tena RG, Kessler PD, Weisman HF, Schulman SP, Blumenthal RS, Jackson DG, Van Dop C. Diminished beta-adrenergic receptor responsiveness and cardiac dilation in hearts of myopathic Syrian hamsters (BIO 53.58) are associated with a functional abnormality of the G stimulatory protein. *Circulation* 1990 ; 81:1341-1352.

Ferguson SSG. Evolving concepts in G-protein coupled receptor endocytosis: the role in receptor desensitization and signaling. *Pharmacol Rev* 2001; 53: 1-24.

Foon KA, Maluish AE, Abrams PG, Wrightington S, Stevenson HC, Alarif A, Fer MF, Overton WR, Poole M, Schnipper EF et al. Recombinant leukocyte A interferon therapy for advanced hairy cell leukemia: therapeutic and immunologic results. *Am J Med* 1986; 80: 351-356.

Frolkis I, Gurevitch J, Yuhas Y, Iaina A, Wollman Y, Chernichovski T, Paz Y, Matsa M, Pevni D, Kramer A, Shapira I, Mohr R. Interaction between paracrine tumor necrosis factor-alpha and paracrine angiotensin II during myocardial ischemia. *J Am Coll Cardiol* 2001; 37 :316-322.

Fuchs B, Breithaupt-Grogler K, Belz GG, Roll S, Malerczyk C, Herrmann V, Spahm-langguth H, Mutschler E. Comparative Pharmacodynamics and pharmacokinetics of candesartan and losartan in man. J Pharm Pharmacol. 2000; 52: 1075-1083.

Fukuda Y, Sassa S. Suppression of cytochrome P4501A1 by interleukin-6 in human HepG2 hepatoma cells. *Biochem Pharmacol* 1994; 47: 1187-1195.

Gabay C, Kushner I. Acute-phase proteins and other systemic responses to inflammation. *N Eng J Med* 1999; 340: 448-484.

Gabay C, Smith MF, Eidlen D, Arend WP. Interleukin-1 receptor antagonist is an acute phase protein. *J Clin Invest* 1997; 99: 2930-2940.

Gajewski TF, Goldwasser E, Fitch FW. Anti-proliferative effect of IFN-gamma in immune regulation. II. IFN-gamma inhibits the proliferation of murine bone marrow cells stimulated with IL-3, IL-4, or granulocyte-macrophage colony-stimulating factor. *J Immunol* 1988; 141: 2635-2642.

Gajewski TF, Fitch FW. Anti-proliferative effect of IFN-gamma in immune regulation I. IFN-gamma inhibits the proliferation of Th2 but not Th1 murine helper T lymphocyte clones. *J Immunol* 1988; 140: 4245-4252.

Gill TS III and Harrington GM. The rat in biomedical research, in Health Benefits of Animal Research, Gay, EI., ED., Foundation for Biomedical Research, 1985.

Godsel LM, Leon JS, Engman DM. Angiotensin concerting enzyme inhibitors and angiotensin II receptor antagonists in experimental myocarditis. *Current Pharmaceutical Design* 2003; 9: 723-735.

Goebeler M, Yoshimura T, Toksoy A, Toksoy A, Ritter U, Brocker EB, Gillitzer R. The chemokine repertoire of human dermal microvascular endothelial cells and tis regulation by inflammatory cytokines. *J Invest Dermatol* 1997; 108: 445-451.

Griselli M, Herbert J, Hutchinson WL, Taylor KM, Sohail M, Krausz T, Pepys MB. C-reactive protein and complement are important mediators of tissue damage in acute myocardial infarction. *J Exp Med* 1999; 190: 1733-1740.

Grisham MB, Johnson GG, Gautreaux MD, Berg RD: Measurement of Nitrate and Nitrite in Extracellular Fluids: A window to Systemic Nitric Oxide Metabolism. *Methods: A Companion to Methods in Enzymology* 1995; 7: 84-90

Gros R, Benovic JL, Tan CM, Feldman RD.G-protein-coupled receptor kinase activity is increased in hypertension. *J Clin Invest* 1997 ; 99:2087-2093.

Gros R, Chorazyczewski J, Meek MD, Benovic JL, Ferguson SS, Feldman RD. G-Protein-coupled receptor kinase activity in hypertension : increased vascular and lymphocyte G-protein receptor kinase-2 protein expression. *Hypertension* 2000; 35:38-42.

Guirguis MS, Jamali F. Disease-Drug Interaction: Reduced Response to Propranolol Despite Increased Concentration in the Rat with Inflammation. *J Pharm Sci* 2003; 92: 1077-1084.

Gurantz D, Cowling RT, Villarreal FJ, Greenberg BH: Tumor necrosis factor-alpha upregulates angiotensin II type 1 receptors on cardiac fibroblasts. *Circ Res* 1999; 85:272-279.

Gutterman JU, Fine S, Quesada J, Horning SJ, Levine JF, Alexanian R, Bernhardt L, Kramer M, Spiegel H, Colburn W, Trown P, Merigan T, Dziewanowski Z. Recombinant leukocyte A interferon: pharmacokinetics, single-dose tolerance, and biologic effects in cancer patients. *Ann Intern Med* 1982; 96: 549-556.

Gutterman JU. Cytokine therapeutics: Lessons from interferon α. *Proc. Natl. Acad. Sci USA*. 1994; 1198-1205.

Hammond HK, Roth DA, Insel PA, Ford CE, White FC, Maisel AS, Ziegler MG, Bloor CM: Myocardial beta-adrenergic receptor expression and signal transduction after chronic volume-overload hypertrophy and circulatory congestion. *Circulation* 1992; 85:269-80.

Handbook of Lab Animal Science Vol II, Animal models. Edited by Per Svendsen and Jann Hau, 1994. Chapter 5: Animal Models of Cardiovascular Research. 43-47.

Hansen PS, Go MF, Varming K, Andersen LP, Genta RM, Graham DY, Nielsen H: Proinflammatory activation of neutrophils and monocytes by *Helicobacter pylori* in patients with different clinical presentations. *Infect Immun* 1999; 67:3171-3174.

Hall SD, Thummel KR, Watkins PB, lown KS, Benet LZ, Paine MF, Mayo RR, Turgeon DK, Baley DG, Fontana RJ, Weighton SA . Molecular and physical mechanisms of first-pass extraction. *Drug Met Disp* 1999; 27: 161-166.

Harbuz MS, Chover-Gonzalez AJ, Biswas S, Lightman SL, Chowdrey HS . Role of central catecholamines in the modulation of corticotrophin-releasing factor mRNA during adjuvant-induced arthritis in the rat. *Br J Rheumatol* 1994; 33: 205-209.

Haworth C, Brennan FM, Chantry D, Turner M, Maini RN, Feldman M. Expression of granulocyte-macrophage colongy-stimulating factor in rheumatology arthritis: regulation by tumor necrosis factor α. *Eur J Immunol* 1991; 21: 2575-2579.

Helica Biosystems Inc. High Sensitivity Rat C-Reactive Protein assay. 96CRP01R, Rat CRP ELISA Kit. 223 E. Imperial Way, Suite 165, Fullerton, CA 92835 http://www.helica.com/life-science-research/kits/

Hinz G, Pohl W. Diagnostic-therapeutic problems on heart involvement in chronic polyarthritis. *J Rheumatol* 1975; 34:39-48.

Hirschfield GM, Pepys MB. C-reactive protein and cardiovascular disease: new insights from an old molecule. *Q J Med* 2003; 96: 793-807.

Horiuchi M, Akishita M, Dzau V. Recent progress in angiotensin II type 2 receptor research in the cardiovascular system. *Hypertension* 1999; 33: 613-621.

Hsieh CS, Macatonia SE, Tripp CS, Wolf SF, O'Garra A, Murphy KM. Development of TH1 $CD4^+$ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* 1993; 260: 547-549.

Hooijberg JH, Broxterman HJ, Kool M, Assaraf YG, Peters GJ, Noordhuis P, Scheper RJ, Borst P, Pinedo HM, Jansen G. Antifolate resistance mediated by the multidrug resistance proteins MRP1 and MRP2. *Cancer Res* 1999; 59: 2532-2535.

Hou J, Schindler U, Henzel WJ, Ho TC, Brasseur M, McKnight SL. An interleukin-4-induced transcription factor: IL-4 Stat. *Science* 1994; 265: 1701-1706.

Huang B, Qin D, El-Sherif N. Early down-regulation of K^+ channel genes and currents in the postinfarction heart. *J Cardiovasc Electrophysiol* 2000 ; 11:1252-1261

Hürlimann D, Forster A, Noll G, Enseleit F, Chenevard R, Distler O, Bechir M, Spieker LE, Neidhart M, Michel BA, Gay RE, Lüscher TF, Gay S, Ruschitzka F. Anti-tumor necrosis factor-α treatment improves endothelial function in patients with rheumatoid arthritis. *Circulation* 2002; 106: 2184-2187.

Iber H, Chen Q, Cheng PY, and Morgan ET. Suppression of CYP2C11 gene transcription by interleukin-1 mediated by NF-kappaB binding at the transcription start site. *Arch Biochem Biophys* 2000; 377: 187-194.

Iber H, Sewer MB, Barclay TB, Mitchell SR, Li T, Morgan ET. Modulation of drug metabolism in infectious and inflammatory diseases. *Drug Met Rev* 1999; 31:29-41.

Inagami T. Molecular biology and signaling of angiotensin receptors: an overview. *J Am Soc Nephrol* 1999; 10:S2-S7.

Ioannou Y, Isenberg DA. Current evidence for the induction of autoimmune rheumatic manifestations by cytokine therapy. *Arthritis Rheum* 2000; 43: 1431-1442.

Ishizuki S, Furuhata K, Kaneta S, Fujihira E. Reduced drug metabolism in isolated hepatocytes from adjuvant arthritic rats. *Res Commun Chem Pathol Pharmacol* 1983; 39: 261-276.

Jiang Y, McCabe D, Aitchison R, Watt I, Genant HK. Relationship of Genent scoring method with Larsen scoring method in randomized, double-blind , placebo controlled trial of recombinant human interleukin-1 receptor antagonist in patients with rheumatoid arthritis. *Arthritis Rheum* 1998; 41:S50.

Jover R, Bort R, Goméz –Léchon MJ, Castell JV . Down-regulation of human CYP 3A4 by the inflammatory signal interleukin-6: molecular mechanism and transcription factors involved. *FASEB J* 2002; 16:1799-1801.

Judy WV, Watanabe AM, Henry DP, Besch HR Jr. Murphy WR, Hockel GM. Sympathetic nerve activity: role in regulation of blood pressure in the spontaneously hypertensive rat. *Circ Res* 1976; 38: 21-29.

Kadoi Y, Saito S, Kawahara F, Nishihara F, Goto F. G-Protein Coupled Receptor Kinase 2 Is Altered during Septic Shock in Rats. *J of Surgical Res* 2002; 108: 69-76.

Kalitsky-Szirtes J, Shayeganpour A, Brocks D, and Piquette-Miller M . Suppression of drug-metabolizing enzymes and efflux transporters in the intestine of endotoxin-treated rats . *Drug Met Disp* 2004; 32: 20-27.

Kaftan AH, Kaftan O . QT intervals and heart rate variability in hypertensive patients. *Jpn Heart J* 2000; 41:173-182.

Kaprielian RR, Dupont E, Hafizi S, Poole-Wilson PA, Khaghani A, Yacoub MH, Severs NJ. Angiotensin II receptor type 1 mRNA is unregulated in atria of patients with end-stage heart failure. *J Mol Cell Cardiol* 1997; 29:2299-2304.

Kaukonen KM, Olkkola KT, Neuvonen PJ. Fluconazole but not itraconazole decreases the metabolism of losartan to E-3174. *Eur J Clin Pharmacol* 1998; 53: 445-449.

Keffer J, Probert L, Cazlaris H, Georgopoulos S, Kaslaris E, Kioussis D, Kollias G. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J* 1991; 10: 4025-403.

Keller E, Bond M, Moravec CS . Progression of left ventricular hypertrophy does not change the sarcoplasmic reticulum calcium store in the spontaneously hypertensive rat heart. *J Mol Cell Cardiol* 1997; 29:461-469.

Khatsenko O. Interactions between nitric oxide and cytochrome p-450 in the liver. *Biochemistry(Moscow)*1998; 63: 833-839.

Kirman I, Whelan RL, Nielsen OH. Infliximab: mechanism of action beyond TNF-alpha neutralization in inflammatory bowel disease. *Eur J Gastroenterol Hepatol*. 2004; 16: 639-641.

Kjeldsen SE, Dahlof B, Devereux RB, Julius S, Aurup P, Edelman J, Beevers G, de Faire U, Fyhrquist F, Ibsen H, Kristianson K, Lederballe-Pedersen O, Lindholm LH, Nieminen MS, Omvik P, Oparil S, Snapinn S, Wedel H. Effects of losartan on cardiovascular morbidity and mortality in patients with isolated systolic hypertension and left ventricular hypertrophy: a Losartan Intervention for Endpoint Reduction (LIFE) substudy. *JAMA* 2002; 288:1491-1498.

Kocarek TA, Schuetz EG, Guzelian PS. Expression of multiple forms of cytochrome P450 mRNAs in primary cultures of rat hepatocytes maintained on matrigel. *Mol Phamacol* 1993; 43:328-334.

Koller-Strametz J, Pacher R, Frey B, Kos T, Woloszczuk W, Stanek B. Circulating tumor necrosis factor-alpha levels in chronic heart failure: relation to its soluble receptor II.

interleukin-6, and neurohumoral variables. *J Heart Lung Transplant* 1998; 17:356-362.

Kompa AR, Gu X-h, Evans BA, Summers RJ. Desensitization of cardiac β-adrenoceptor signaling with heart failure Produced by myocardial Infarction in the rat. Evidence for the role of Gi but not Gs or phosphorylating proteins. *J Mol Cell Cardiol* 1999; 31:1185-1201.

Kopf D, Waldherr R, Rettig R. Source of kidney determines blood pressure in young transplanted rats. *Am J Physiol.* 1993; 265(1 Pt 2): F104-F111.

Krämer C, Sunkomat J, Witte J, Luchtefeld M, Walden M, Schmidt B, Böger RH, Forssmann WG, Drexler H, Schieffer B. Angiotensin II receptor-independent antiinflammatory and antiaggregatory properties of losartan: role of the active metabolite EXP3179. *Circ Res* 2002; 90: 770-776.

Kraemer MJ, Frukawa C, Koup JP, and Shapiro G. Altered theophylline clearance during an influenza outbreak. *Pediatrics* 1982; 69:476-480.

Krause A, Steitz A, von Wichert P, Baerwald C. Influence of cytokines on the density of beta 2-adrenergic receptors on peripheral blood mononuclear cells in vitro. *Cytokine* 1995; 7: 273-276.

Kuby Janis (1997) Immunology. 3rd ed. (WH Freeman and Company) Part I Introduction. P 3-82.

Kulmatycki KM, Abouchehade K, Sattari S, Jamali F. Drug-disease interactions: reduced β-adrenergic and potassium channel antagonist activities of sotalol in the presence of acute and chronic inflammatory conditions in the rat. *British J of Pharmacol* 2001; 133: 286-294.

Kupershmidt S, Yang IC, Hayashi K, Wei J, Chanthaphaychith S, Petersen CI, Johns DC, George AL Jr, Roden DM, Balser JR . The IKr drug response is modulated by KCR1 in transfected cardiac and noncardiac cell lines. *FASEB J* 2003; 17:2263-2265.

Kushner I, Broder ML, Karp D. Control of the acute phase response. Serum C-reactive protein kinetics after acute myocardial infarction. *J Clin Invest* 1978; 61: 235-242.

Kurzrock R, Gutterman JU, Talpaz M. The molecular genetics of Philadelphia chromosome-positive leukemias. *N. Engl. J Med.* 1988; 319: 990-998.

Lagrand WK, Visser CA, Hermens WT, Niessen HW, Verheugt FW, Wolbink GJ, Hack CE. C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? *Circulation* 1999; 100: 96-102.

Lanchbury JS. The HLA association with rheumatoid arthritis. *Clin Exp Rheumatol* 1992; 10: 301-304.

Leavitt RD, Ratanatharathorn V, Ozer H, Ultmann JR, Portlock C, Myers JW, Kisner D, Norred S, Spiegel RJ, Bonnem EM. Alpha-2b interferon in the treatment of Hodgkin's disease and non-Hodgkin's lymphoma. *Semin Oncol* 1987; 14: 18- 3.

Lepore L, Pennesi M, Saletta S, Perticarari S, Presani G, Prodan M. Study of IL-2, IL-6, TNF alpha, IFN gamma and beta in the serum and synovial fluid of patients with juvenile chronic arthritis. *Clin Exp Rheumatol* 1994; 12: 561-565.

Li X, Jiang W. Electrical remodeling of membrane ionic channels of hypertrophied ventricular myocytes from spontaneously hypertensive rats. *Chinese Med J* 2000; 113: 584-587.

Li L, Xia Y, Nguyen A, Lai YH, Ferg L, Mosmann TR, Lo D. Effects of Th2 cytokines on chemokine expression in the lung: IL-13 potently induces eotaxin expression by airway epithelial cells. *J Immunol* 1999; 162: 2477-2487.

Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation* 2002; 105: 1135-1143.

Lieu L, Zhao SP. The changes of circulating tumor necrosis factor levels in patients with congestive heart failure influenced by therapy. *Int J Cardiol* 1999; 69:77-82

Lindholm LH, Ibsen H, Dahlöf B, Devereux RB, Beevers G, Faire U, Gyhrquist F, Julius S, Kjeldsen SR, Kristiansson K, Lederballe-Pederson O, Nieminen MS, Omvik P, Oparil S, Wedel H, Aurup P, Edelman J, Snapinn S. Cardiovascular morbidity and mortality in patients with diabetes in the Losartan Intervention For Endpoint reduction in hypertension study (LIFE): a randomized trial against atenolol. *The Lancet* 2002; 359: 1004-1010.

Lindmark E, Diderholm E, Wallentin L, Siegbahn A. Relationship between interleukin 6 and mortality in patients with unstable coronary artery disease: effects of an early invasive or noninvasive strategy. *JAMA* 2001; 286:2107-2113.

Lincoln J, Hoyle HVH, Burnstock G. Nitrix oxide in health and disease. Cambridge University Press 1997.

Little R, White MR, Hartshorn KL. Interferon-alpha enhances neutrophil respiratory burst responses to stimulation with influenza A virus and FMLP. *J Infect Dis* 1994; 170:802-810.

Liuzzo G, Biasucci LM, Gallimore JR, Grillo RL, Rebuzzi AG, Pepys MB, Maseri A. The prognostic value of C-reactive protein and serum amyloid A protein in severe unstable angina. *N Engl J Med* 1994; 331: 417-424.

Lo MW, Goldberg MR, McCrea JB, Lu H, Furtek CI, Bjornsson TD. Pharmacokinetics of losartan, an angiotensin II receptor antagonist, and its active metabolite EXP-3174 in humans. *Clin Pharmacol Ther* 1995; 58: 641-649.

Lombardi MS, Kavelaars A, Cobelens PM, Schmidt RE, Schedlowski M, Heijnen CJ. Adjuvant arthritis induces down-regulation of G protein-coupled receptor kinases in the immune system. *Immunol* 2001; 166:1635-1640.

Lombardi MS, Kavelaars A, Heijnen C. Role and modulation of G protein-coupled receptor signaling in inflammatory processes. *Crit Rev Immunol* 2002; 22: 141-163.

Lombardi MS, Kavelaars A, Schedlowski M, Bijlsma JW, Okihara KL, Van de Pol M, Ochsmann S, Pawlak C, Schmidt RE, Heijnen CJ. Decreased expression and activity of G-protein-coupled recep tor kinases in peripheral blood mononuclear cells of patients with rheumatoid arthritis. *FASEB J.* 1999;13: 715-725.

Lombardi MS, Van den Tweel E, Kavelaars A, Groenendaal F, van Bel F, Heijnen CJ. Hypoxia/ischemia modulates G protein-coupled receptor kinase 2 and beta-arrestin-1 levels in the neonatal rat brain. *Stroke* 2004 ; 35:981-986.

Loubaris N, Cros G, Serrano JJ, Boucard M. Circadian and circannual variation of the carrageenin inflammatory effect in rat. *Life Sci.* 1983; 32:1349-1354.

Lu HR, Yu F, Dai DZ, Remeysen P, Clerck FD. Reduction in QT dispersion and ventricular arrhythmias by ischaemic preconditioning in anaesthetized, normotensive and spontaneously hypertensive rats. *Fundam Clin Pharmacol* 1999; 13: 445-454.

MacNaul KL, Chartrain N, Lark M, Tocci MJ, Hutchinson JI. Discoordinate expression of stromelysin, collagenase, and tissue inhibitor of metalloproteinases-1 in rheumatoid human synovial fibroblasts: synergistic effects of interleukin-1 and tumor necrosis factor-alpha on stromelysin expression. *J Biol Ch*em 1990; 265: 17238-17245.

Manzi S, Meilahn EN, Rairie JE, Conte CG, Medsger TA Jr, Jansen McWilliams L, D'Agostino RB, Kuller LH. Age-specific incidence rates of myocardial infarction and angina in women with systemic lupus erythematosus: comparison with the Framingham Study. *Am J Epidemiol* 1997; 145: 408-415.

Marrero MB, Schleffer B, Paxton WG, Heerdt L, Berk BC, Delafontaine P, Bernstein KE. Direct stimulation of Jak/STAT pathway by the angiotensin II AT_1 receptor. *Nature* 1995; 375:247-250.

Malaguarnera M, Di Fazio I, Restuccia S, Pistone G, Ferlito L, Rampello L. Interferon alpha-induced depression in chronic hepatitis C patients: comparison between different types of interferon alpha. *Neuropsychobiology*. 1998; 37: 93-97.

Martin J, Krum H. Role of valsartan and other angiotensin receptor blocking agents in the management of cardiovascular disease. *Pharmacol Res* 2002; 46: 203-212.

Maury CP. Monitoring the acute phase response: comparison of tumor necrosis factor (cachectin) and C-reactive protein responses in inflammatory and infectious diseases. *J Clin Pathol* 1989; 42: 1078-1082.

Mayo PR, Skeith K, Russell AS, Jamali F. Decreased Dromotropic response to verapamil despite pronounced increased drug concentration in rheumatoid arthritis. *Br J Clin Pharmacol* 2000; 50: 605-613.

McEntegart A, Capella HA, Creran D, Rumley A, Woodward M, Lowe GD. Cardiovascular risk factors, including thrombotic variables, in a population with rheumatoid arthritis. *Rheumatology (Oxford)* 2001; 40: 640-644.

McCrea JB, Cribb A, Rushmore T, Osborne B, Gillen L, Lo M-W, Waldman S, Bjornsson T, Spielberg S, Goldberg MR. Losartan, an orally active angiotensin (AT1) receptor antagonist: a Review of its efficacy and safety in essential hypertension. *Pharmacol Ther* 1997; 4: 181-194.

McCrea JB, Cribb A, Rushmore T, Osborne B, Gillen L, Lo M-W, Waldman S, Bjornsson T, Spielberg S, Goldberg MR. Phenotypic and genotypic investigations of a healthy volunteer deficient in the coversion of losartan to its active metabolite E-3174. *Clin Pharmacol Ther* 1999; 65; 348-352.

McIntyre M, Caffe SE, Michalak RA, Reid JL. Losartan, an orally active angiotensin (AT_1) Receptor Antagonist: A review of its efficacy and safety in essential hypertension. *Pharmacol Ther* 1997: 181-194.

Meij JT. Regulation of G protein function: implications for heart disease. *Mol Cell Biochem* 1996; 157: 31-38.

Meyer P, Gaay RP, DeMendoca M. Ion transport systems in hypertension. editors In Genest J, Kuchel I, hamet P, Cantin M : 1983 "Hypertension" pp. 108-116.

Miyata M, Ohira H, Sasajima T, Suzuki S, Ito M, Sato Y, Kasukawa R. Significance of low mRNA levels of interleukin-4 and -10 in mononuclear cells of the synovial fluid of patients with rheumatoid arthritis. *Clinical Rheumatology* 2000; 19: 365-370.

Mohler KM, Torrance DS, Smith CA, Goodwin RG, Stremler KE, Fung VP, Madani H, Widmer MB. Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoximia and function simultaneously as both TNF carriers and TNF antagonists. *J Immunol* 1993; 151: 1548-1561.

Mold C, Gewurz H, Du Clos TW. Regulation of complement activation by C-reactive protein. I*mmunopharmacology* 1999; 42: 23-30.

Moncada S, Palmer RMJ, Higgs EA. Nitric oxide. Physiology, pathology and pharmacology. *Pharmacol Rev* 1991; 43: 109-142.

Morris RB, Imber MJ, Heinsimer JA, Hlatky MA, Reimer K. Rheumatoid arthritis and coronary arteritis. *Am J Cardiol* 1986; 57: 689-690.

Moreland LW, Baumgartner SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL, Ettlinger RE, Cohen S, Koopman WJ, Mohler K, Widmer MB, Blosch CM. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p7)-Fc fusion protein . *N Engl J Med* 1997; 337: 141-147.

Moreland LW, Schiff MH, Baumgartner SW, Tindall EA, Fleischmann RM, Bulpitt KJ, Weaver AL, Keystone EC, Furst DE, Mease PJ, Ruderman EM, Horwitz DA, Arkfeld DG, Garrison L, Burge DJ, Blosch CM, Lange ML, McDonnell ND, Weinblatt ME. Etanercept therapy in rheumatoid arthritis: a randomized controlled trial. *Ann Intern Med* 1999; 130: 478-486.

Morgan JM, Palmisano M, Piraino A, Hirschhorn W, Spencer S, Prasad PP, Ortiz M, Lloyd P. The effect of valsartan on the angiotensin II pressor response in healthy normotensive male subjects. *Clin Pharmacol Ther* 1997; 61: 35-44.

Morgan ET. Regulation of cytochromes P450 During Inflammation and Infection. *Drug Met Rev* 1997; 29: 1129-1188.

Morgan ET, Sewer MB, Iber H, Gonzalez FJ, Lee Y-U, Tukey RH, Okino S, Vu T, chen Y-U, Sidhu JS, Omiecinski CJ. Physiological and pathophysiological regulation of cytochrome P450. Anthony Y.H.Lu Commemorative Issue. *Drug Met Disp* 1998; 26: 1232-1240.

Moriguchi Y, Matsubara H, Mori Y, Murasawa S, Masaki H, Maruyama K, Tsutsumi Y, Shibasaki Y, Tanaka Y, Nakajima T, Oda K, Iwasaka T. Angiotensin II-induced transactivation of epidermal growth factor receptor regulates fibronectin and transforming growth factor β synthesis via transcriptional and post-transcriptional mechanisms. *Circ Res* 1999; 84: 1073-1084.

Motojima M, Kakuchi J, Yoshioka T. Association of TGF-beta signaling in angiotensin II-induced PAI-1 mRNA upregulation in mesangial cells: role of PKC. *Biochim Biophys Acta.* 1999; 1449: 217-226.

Muller DN, Dechend R, Mervaala EM, Park JK, Schmidt F, iebeler A, Theuer J, Breu V, Ganten D, Haller H, Luft FC. NF-kappaB inhibition ameliorates angiotensin II-induced inflammatory damage in rats. *Hypertension* 2000; 35: 193-201.

Müller P, Flesch G, de Gasparo M, Gasparini M, Howard H. Pharmacokinetics and Pharmacodynamic effects of the angiotensin II antagonist valsartan at steady state in healthy normotensive subjects *Eur J Clin Pharmacol* 1997; 52: 441-449.

Mutru O, Laakso M, Isomaki H, Koota K. Ten year mortality and causes of death in patients with rheumatoid arthritis. *Br Med J (Clin Res Ed)* 1985; 290:1797-1799.

Mutru O, Laakso M, Isomaki H, Koota K. Cardiovascular mortality in patients with rheumatoid arthris. *Cardiology* 1989; 76: 71-77.

Myllykangas-Luosujarvi RA, Aho K, Kautianinen H, Isomaki H. Cardiovascular mortality in females with rheumatoid arthritis. *J Rheumatolo* 1995; 22: 1065-1067.

Myllykangas-Luosujarvi RA, Aho K, Kautianinen H, Isomaki H. Shortening of life span and causes of excess mortality in a population-based series of subjects with rheumatoid arthritis. *Clin Exp Rheumatol* 1995; 13: 149-153

Myllykangas-Luosujarvi RA. Aho K. Isomaki HA. Mortality in rheumatoid arthritis. [Review] *Seminars in Arthritis & Rheumatism* 1995; 25:193-202.

Nagatsu T, Kato T, Numata Y, Keiko I, Umezawa H. Serum dopamine beta-hydroxylase activity in developing hypertensive rats. *Nature* 1974; 251: 630-631.

Nathan C, Xie Q. Regulation of biosynthesis of nitric oxide. *J Biol Chem* 1994; 269: 13725-13728.

Navalkar S, Parthasarathy S, Santanam N, Khan BV. Irbesartan, an angiotensin type 1 receptor inhibitor, regulates markers of inflammation in patients with premature atherosclerosis. *J Am Coll Cardiol* 2001 ; 37: 440-444.

Nawroth PP, Rank I, Handley D, Cassimeris J, Chess L, Stern D. Tumor necrosis factor/cachectin interacts with endothelial cell receptors to induce release of interleukin I. *J Exp Med* 1986; 163: 1363-1375.

Neer EJ. Heterotrimetic G proteins: Organizers of transmembrane signals. *Cells* 1995; 80: 249-257.

Nesher G, Ruchlemer R. Alpha-interferon-Induced Arthritis: Clinical Presentation, Treatment, and Prevention. *Semin Arthritis Rheum* 1998; 27: 360-365.

Nickoloff BJ, Naidu Y. Perturbation of epidermal barrier function correlates with initaition of cytokines cascade in human skin. *J Am Acad Dermatol* 1994; 30: 535-546.

Niimi R, Nakamura A, Yanagawa Y. Suppression of endotoxin-induced renal tumor necrosis factor-alpha and interleukin-6 mRNA by renin-angiotensin system inhibitors. *Jpn J Pharmacol* 2002; 88: 139-145.

Numaguchi H, Mullins FM, Johnson JP Jr, Johns DC, Po SS, Yang IC, Tomaselli GF, Balser JR. Probing the interaction between inactivation gating and Dd-sotalol block of HERG. *Circ Res*. 2000; 87:1012-1018.

Oberg K, Norheim I, Lind E, Alm G, Lundqvist G, Wide L, Jonsdottir B, Magnusson A, Wilander E. Treatment of malignant carcinoid tumors with human leukocyte interferon: Long-term results. *Cancer Treat Rep* 1986; 70: 1297-1304.

Ohmori Y, Hamilton TA. IL-4-induced STAT6 suppresses IFN-gamma-stimulated STAT1-dependent transcription in mouse macrophages. *J Immunol* 1997; 159: 5474-5482.

Okamoto K, Aoki K. Development of a strain of spontaneously hypertensive rats. *Jpn Circ J* 1963; 27: 282-293.

Okamoto K. Spontaneous hypertension in rats. *Int Rev Exp Pathol*. 1969; 7: 227-270.

Okamura A, Rakugi H, Ohishi M, Yanagitani Y, Takiuchi S, Moriguchi K, Fennessy PA, Higaki J, Ogihara T. Upregulation of renin-angiotensin system during differentiation of monocytes to macrophages. *J Hypertension* 1999; 17: 537-545.

Okin PM, Devereux RB, Jern S, Kjeldsen SE, Julius S, Nieminen MS, Snapinn S, Harris KE, Aurup P, Edelman JM, Dahlof B. Losartan Intervention for Endpoint reduction in hypertension Study Investigations. Regression of electrocardiographic left ventricular hypertrophy by losartan versus atenolol: The Losartan Intervention for Endpoint reduction in Hypertension (LIFE) Study. *Circulation* 2003; 108: 684-690.

Parronchi P, De Carli M, Manetti R, Simonelli C, Sampognaro S, Piccinni MP, Macchia D, Maggi E, Del Prete G, Romagnani S. IL-4 and IFN (alpha and gamma) exert opposite regulatory effects on the development of cytolytic potential by Th1 or Th2 human T cell clones. *J Immunol.* 1992; 149: 2977-2983.

Pascaud C, Garrigos M, Orlowski S. Multidrug resistance transporter P-glycoprotien has distinct but interacting binding sites for cytotoxic drugs and reversing agents. *Biochem J* 1998; 333: 351-358.

Pascual RM, Billington CK, Hall IP, Panettieri RA, Fish JE, Peters SP, Penn RB. Mechanisms of cytokine effects on G protein-coupled receptor-mediated signalling in airway smooth musle. *Am J Physiol Lung Call Mol Physiol* 2001; 281: L1425-1435.

Pearson TA, Menasah GA, Alexander RW, Anderson JL, Cannon RO 3rd, Criqui M, Fadl YY, Fortmann SP, Hong Y, Myers GL, Rifai N, Smith SC Jr, Taubert K, Tracy RP, Vinicor F; Centers for Disease and Prevention; American Heart Association. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A Statement For Healthcare Professionals From The Centers For Disease Control And Prevention And The American Heart Association. *Circulation* 2003; 107: 499-511.

Peeters ACTM, Netea MG, Kullberg BJ, Thien T, Van der Meer JWM. The effect of renin-angiotensin system inhibitors on pre- and anti- inflammatory cytokine production. *Immunology* 1998; 94: 376-379.

Peeters AC, Netea MG, Janssen MC, Kullberg BJ, Van der Meer JW, Thien T. Pro-inflammatory cytokines in patients with essential hypertension. *Eur J Clin Invest* 2001; 31: 31-36.

Peng JF, Gurantz D, Tran V, Cowling RT, Greenberg BH. Tumor Necrosis Factor α-Induced AT1 Receptor Upregulation Enhances Angiotensin II Mediated Cardiac Fibroblast Responses That Favor Fibrosis .*Circ Res* 2002; 91: 1119-1126.

Penn RB, Shaver JR, Zangrilli JG, Pollice M, Fis JE, Peters SP, Benovic JL. Effects of inflammation and acute beta-agonist inhalation on beta 2-AR signaling in human airways. *Am J Physiol* 1996; 271: L601-L608.

Perron A, Chen Z-G, Gingras D, Dupré DJ, Staňková J, Rola-Pleszcynski M. Agonist-independent desensitization and internalization of the human platelet-activating factor receptor by coumermycin-gyrase B induced Dimerization. *J Biol Chem* 2003; 278: 27956-27965.

Pestka S, Langer JA., Zoon KC, and Samuel CR. Interferons and their actions. *Annu Rev Biochem* 1987; 56: 727-777.

Petrovsky N, McNair P, Harrison LC. Diurnal rhythms of pro-inflammatory cytokines: regulation by plasma cortisol and therapeutic implications. *Cytokine.* 1998; 10: 307-312.

Phillips MI, Kagiyama S. Angiotensin II as a pro-inflammatory mediator. *Curr Opin Investig Drugs*. 2002 ; 3:569-577.

Pietila KO, Harmoinen AP, Jokiniitty J, Pasternack AI. Serum C-reactive protein concentration in acute myocardial infarction and its relationship to mortality during 24 months of follow-up in patients under thrombolytic treatment. *Eur Heart J* 1996; 17:1345-1349.

Pincus T, Callahan LF: Taking mortality in rehmatoid arthritis seriously-predictive markers, socioeconomic status and comorbidity. *J Reumatol* 1986; 13: 841-845.

Piquette-Miller M, Jamali F. Influence of severity of inflammation on the disposition kinetics of propranolol enantiomers in ketoprofen-treated and untreated adjuvant arthritis. *Drug Metab Dispos* 1995; 23: 240-245.

Plant MJ, Williams AL, O'Sullivan MM, Lewis PA, Coles EC, Jessop JD. Relationship between time-integrated C-reactive protein levels and radiologic progression in patients with rheumatoid arthritis. *Arthritis Rheum.* 2000; 43:1473-1477.

Pond AL, Nerbonne JM. ERG proteins and functional cardiac Ikr channels in rat, mouse, and human heart. *Cardiovasc Med* 2001; 11: 286-294.

Popa C, Netea MG, Radstake T, Van Der Meer JW, Stalenhoef AF, Van Riel PL, Barrera P. Influence of anti-TNF treatment on the cardiovascular risk factors in patients with active rheumatoid arthritis. *Ann Rheum Dis.* Online First July 1 2004. doi: 10.1136/ard.2004023119.

Porter JP. Beta-Adrenoceptor modulation of renin response to short-term reductions in pressure in young SHR. *Am J Physiol.* 1992; 263: R405-411.

Prasad A, Koh KK, Schenke WH, Mincemoyer R, Csako G, Fleischer TA, Brown M, Selvaggi TA, Quyyumi AA. Role of angiotensin II type 1 receptor in the regulation of cellular adhesion molecules in atherosclerosis. *Am Heart J* 2001; 142: 248-253.

Prior P, Symmons DP, Scott DL, Brown R, Hawkins CF. Cause of death in rheumatoid arthritis. *Br J Rheumatol* 1984; 23: 92-99.

Proulx M, du Souich P. Inflammation-induced decrease in hepatic cytochrome P450 in conscious rabbits is accompanied by an increase in hepatic oxidative stess. Res Commin *Mol Pathol Pharmacol* 1995; 87:221-236.

Putney Jr. JW. Excitement about calcium signaling in inexcitable cells. *Science* 1993; 262: 676-678.

Quesada JR, Alexanian R, Hawkins M, Barlogie B, Borden E, Itri L, Gutterman JU. Treatment of multiple myeloma with recombinant alpha-interferon. *Blood* 1986; 67: 275-278.

Raasch W, Bartels T, Schwartz C, Hauser W, Rutten H, Dominiak P. Regression of ventricular and vascular hypertrophy: are there differences between structurally different angiotensin-converting enzyme inhibitors? *J Hypertens* 2002; 20: 2495-2504.

Rahmani MA, DeGray G, David V, Ampy FR, Jones L.Comparison of calcium import as a function of contraction in the aortic smooth muscle of Sprague-Dawley, Wistar Kyoto and spontaneously hypertensive rats. *Front Biosci.* 1999 ; 4:D408-415.

Rathanaswami P, Hachicha M, Sadick M, Schall TJ, McColl SR. Expression of the cytokine RANTES in human rheumatoid synovial fibroblasts. Differential regulation of RANTES and interleukin-8 genes by inflammatory cytokines. *J Biol Chem* 1993; 268: 5834-5839.

Renton KW. Hepatic Drug Metabolism and Immunostimulation. *Toxicology* 2000; 142: 173-178

Renton KW. Alteration of drug biotransformation and elimination during infection and inflammation. *Pharmacol Ther* 2001;92: 147-163.

Ridker PM. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation* 2001; 103: 1813-1818.

Ridker PM, Buring JE, Shih J, Matias M, Hennekens CH. Prospective study of C-reactive protein and the risk of future cardiovascular events among apparently healthy women. *Circulation* 1998; 98: 731- 733.

Ridker PM, Cushman M, Stampfer MJ,Tracy RP, Hennekens CH. Inflammation, aspirin and the risk of cardiovascular disease in apparently healthy men. *N Engl J Med.* 1997; 336: 973-979.

Ritter MA, Furtek CL, Lo MW. An improved method for the simultaneous determination of losartan and its major metabolite, EXP3174, in human plasma and urine by high-performance liquid chromatography with fluorescence detection. *J Pharm Biomed Anal.* 1997; 15: 1021-1029.

Robinson D, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of $CD4^+$ T cells, increased TH2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in patients with atopic asthma. *J Allergy Clin Immunol* 1993; 92: 313-324.

Rosenson RS, Koenig W. High-sensitivity C-reactive protein and cardiovascular risk in patients with coronary heart disease. *Curr Opin Cardiol* 2002; 17: 325-331

Ruiz-Ortega M, Lorenzo O, Rupérez M, König S, Wittig B, Egido J. Angiotensin II activates nuclear transcription factor κB through AT1 and AT2 in vascular smooth muscle cells. Molecular mechanisms. *Circ Res* 2000; 86: 1266-1272.

Ruiz-Ortega M, Lorenzo O, Rupérez M, Esteban V, Suzuki Y, Mezzzano S, Plaza JJ, Egido J. Role of the renin-angiotensin system in vascular diseases. Expanding the field. *Hypertension* 2001; 38: 1382-1387.

Sadoshima J. Cytokine actions of angiotensin II. *Circ Res* 2000; 86: 1187-1189.

Sadoshima J. Novel AT_1 Receptor-Independent Functions of Losartan. *Circ Res* 2002; 90: 754-756.

Salehzada T, Silhol M, Steff AM, Lebleu B, Bisbal C. 2'5'-Oligoadenylate-dependent RNase L is a dimmer of regulatory and catalytic subunits. *J Biol Chem* 1993; 268: 7733-7740.

Sen GC, Lengyel P. The interferon system. A bird's eye view of its biochemistry. *J Biol Chem.* 1992; 267: 5017-5020.

Sano T, Tanaka A, Namba M, Nishibori Y, Nishida Y, Kawarabayashi T, Fukuda D, Shimada K, Yoshikawa J. C-reactive protein and lesion morphology in patients with acute myocardial infarction. *Circulation*. 2003; 108: 282-285.

Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "High-Grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis (Review). *Circulation* 2003; 108:2957-2963.

Sattari S, Dryden W, Jamali F. The effect of inflammation on the binding of cardioactive agents to rat cardiac cell membrane. 17th annual meeting of American Association of Pharmaceutical Scientists, Toronto, Ontario, Canada, November 10-14, 2002.

Sattari S, Dryden WF, Eliot LA, Jamali F. Despite increased plasma concentration, inflammation reduces potency of calcium channel antagonists due to lower binding to the rat heart. *Br J Pharmacol* 2003; 139: 945-954.

Saito M, Ishimitsu T, Minami J, Ono H, Ohrui M, Matsuoka H. Relations of plasma high-sensitivity C-reactive protein to traditional cardiovascular risk factors. *Atherosclerosis* 2003;167: 73-79.

Samaras SC, Deitz N. Physiopathology and detoxification of pentobarbital sodium. *Fed Am Soc Exp Biol* 1953; 12: 400.

Sayeski PP, Showkat Ali M, Semeniuk DJ, Doan TN, Bernstein KE. Angiotensin II signal transduction pathways. *Regulatory Peptides* 1998; 78:19-29

Schindler R, Dinarello CA, Koch KM. Angiotensin-converting enzyme inhibitors suppress synthesis of tumour necrosis factor and interleukin 1 by human peripheral blood mononuclear cells. *Cytokine* 1995; 7: 526-533.

Schindler R, Mancilla J, Endres S, Ghorbani R, Clark SC, Dinarrello CA: Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human mononuclear cells: IL-6 suppresses IL-1 and TNF. *Blood* 1990; 75: 40-47.

Schulze-Koops H, Kalden JR. The balance of Th1/Th2 cytokines in rheumatoid arthritis. Best Practice and Research *Clin Rheumatol* 2001; 15: 677-691.

Schwentker A, Vodovotz , Weller R, Billiar TR. Nitric oxide and would repair: role of cytokines? *Nitric Oxide* 2002; 1-10

Schumacher H, Klippel J, Koopman W, eds. *Primer of the rheumatic diseases* 10th edn. Arthritis Foundation. William Byrd Press Richmond VA., p. 328, 1993.

Scott DL, Symmons DP, Coulton BL, Popert AJ. Long-term outcome of treating rheumatoid arthritis: results after 20 years. *Lancet* 1987; 1: 1108-1111.

Seder RA, Gazzinelli R, Sher A, Paul WE. Interleukin 12 acts directly on $CD4^+$ T cells to enhance priming for interferon gamma production and diminishes interleukin 4 inhibition of such priming. *Proc Natl Acad Sci* USA 1993; 90: 10188-10192.

See S. Angiotensin II receptor blockers for the treatment of hypertension. *Expert Opin. Pharmacother* 2001; 2: 1795-1804.

Sekino K, Kubota T, Okada Y, Yamada Y, Ymamoto K, Horiuchi R, Kimura K, Iga T. Effect of the single CYP2C9*3 allele on pharmacokinetics and pharmacodynamics of losartan in healthy Japanese subjects. *Eur J Clin Pharmacol* 2003; 59:589-592.

Sewer MB, Barclay TB, Morgan ET. Differential inductive and suppressive effects of endotoxin and particulate irritants on hepatic and renal cytochrome p-450 expression. *J Pharmcol Exp Ther* 1997; 280: 1445-1454.

Shedlofsky SI, Israel BC, McClain CJ, Hill DB, Blouin RA. Endotoxin administration to humans inhibits hepatic cytochrome P450-mediated drug metabolism. *J Clin Invest* 1994; 94: 2209-2214.

Shi HZ, Deng JM, Xu H, Nong ZX, Xiao CQ, Liu ZM, Qin SM, Jiang HX, Liu GN, Chen YQ. Effect of inhaled interleukin-4 on airway hyperreactivity in asthmatics. *Am J Respir Crit Care Med* 1998; 15: 1818-1821.

Schmidt B, Schieffer B. Angiotensin II AT1 Receptor Antagonists. Clinical Implications of Active Metabolites. *J Med Chem* 2003; 46: 2261-2271.

Schumacher H, Klippel J, Koopman W. Primer of the rheumatic diseases 10th edn. Arthritis foundation. William Byrd Press Richmond VA, p. 328 1993.

Sica DA, Lo MW, Shaw WC, Keane WF, Gehr TWB, Halstenson CE, Lipschutz K, Furtek CI, Ritter MA, Shahinfar S. The Pharmacokinetics of losartan in renal insufficiency. *J Hypertens* 1995; 13: S49-S51.

Siegel J, Rent R, Gewurz H. Interactions of C-reactive protein with the complement system. I. Protamine-induced consumption of complement in acute phase sera. *J Exp Med* 1974; 140: 631-647.

Sims JE, Gayle MA, Slack JL, Alderson MR, Bird TA, Giri JG, Colotta F, Re F, Mantovani A, Shanebeck Kl, Grabstein KH, Dower SK. Interleukin 1 signaling occurs exclusively via the type I receptor. *Proc Natl Acad Sci USA* 1993; 261: 472-475.

Singh BN, Deedwania P, Nademanee K, Ward A, Sorkin EM. Sotalol. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic use. *Drugs* 1987; 34: 311-349. Review.

Sioufi A, Marfil F, Jaouen A, Cardot JM, Godbillon J, Ezzet F, Lloyd P. The effect of age on the pharmacokinetics of valsartan. *Biopharmaceutics and Drug Dispos.* 1998; 19: 237-244.

Sioufi A, Marfil F, Godbillon J. Automated determination of an angiotensin II receptor antagonist, CGP 48 933, in plasma by high-performance liquid chromatography. *J Liq Chromatogr.* 1994; 17: 2179-2186

Smirk FH, Hall WH. Inherited hypertension in rats. Nature 1958; 182: 727-728.

Soldner A, Benet LZ, Mutschler E, Christians U. Active transport of the angiotensin-II antagonist losartan and its main metabolite EXP 3174 across MDCK-MDR1 and Caco-2 cell monolayers. *Brit J Pharmacol* 2000; 129: 1235-1243.

Solomon DH, Karlson EW, Rimm EB, Cannuscio CC, Mandl LA, Manson JE, Stampfer MJ, Curhan GC. Cardiovascular morbidity and mortality in women diagnosed with rheumatoid arthritis.*Circulation* 2003; 107: 1303-1307

Sornasse T, Larenas PV, Davis KA, de Vries JE, Yssel H. Differentiation and stability of T helper 1 and 2 cells derived from naive human neonatal CD4$^+$ T cells, analyzed at the single-cell level. *J Exp Med* 1996; 184: 473-483.

Spellberg B, Edwards JR. Type1/Type 2 Immunity in Infectious Diseases. *Clin Inf Dis* 2001; 32: 76-102.

Stearns RA, Chakravarty PK, Chen R, Chiu SHL. Biotransformation of losartan to its active carboxylic acid metabolite in human liver microsomes. *Drug Metab Dispos* 1995; 23: 207-215.

St Lezin E, Simonet L, Pravenec M, Kurtz TW. Hypertensive strains and normotensive 'control' strains. How closely are they related? *Hypertension* 1992; 19: 419-424.

Sukhai M, Yong A, Pak A, and Piquette-Miller M. Decreased expression of P-glycoprotein in interleukin-1β and interleukin-6 treated rat hepatocytes. *Inflamm Res* 2000; 50:362-370.

Sung KC, Suh JY, Kim BS, Kang JH, Kim H, Lee MH, Park JR, Kim SW. High sensitivity c-reactive protein as an independent risk factor for essential hypertension. *AJH* 2003; 16: 429-433.

Svenson M, Nedergaard S, Heegaard PMH, Whisenant TD, Arend WP, Bendtzen K. Differential binding of human interleukin-1 (IL-1) receptor antagonist to natural and recombinant soluble and cellular IL-1 type receptors. *Eur J Immunol* 1995; 25: 2842-2850.

Swain SL, McKenzie DT, Weinberg AD, Hancock W. Characterization of T helper 1 and 2 cell subsets in normal mice. Helper T cells responsible for IL-4 and IL-5 production are present as precursors that require priming before they develop into lymphokine-secreting cells. *J Immunol* 1988; 141: 3445-3455.

Talpaz M, Kantarjian HM, McCredie K, Trujillo JM, Keating MJ, Gutterman JU. Hematologic remission and cytogenetic improvement induced by recombinant human interferon alpha in chronic myelogenous leukemia. *N Engl J Med* 1986; 314: 1065-1069.

Tanaka M, Tsuchida S, Imai T, Fujii N, Muyazaki H, Ichiki T, Naruse M, Inagemi T. Vascular response to angiotensin II is exaggerated through an upregulation of AT1 receptor in AT2 knockout mice. *Biochem Buihy Res Commun* 1999; 258: 194-198.

Tak PP, Taylor PC, Breedveld FC Smeets TJ, Daha MR, Kluin PM, Meinders AE, Maini RN. Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor α monoclonal antibody treatment in patients with rheumatoid arthritis. *Arthritis Rheum* 1996; 39: 1077-1081.

Takeichi N, Suzuki K, Kobayashi H. Characterization of immunological depression in spontaneously hypertensive rats. *Eur J Immunol.* 1981; 11: 483-487.

Tanase H. Genetic control of blood pressure in spontaneously hypertensive rats (SHR). *Jikken Dobutsu* 1979; 28: 519-530.

Teran LM, Mochizuki M, Bartels J, Valencia EL, Nakajima T, Hirai K, Schroder JM. Th1-and Th2-type cytokines regulate the expression and production of eotazin and RANTES by human lung fibroblasts. *Am J Respir Cell Mol Biol* 1999; 20: 777-786.

Terashima T, Amakawa K, Matsumaru A, van Eeden S, Hogg JC, Yamaguchi K. BAL Induces an Increase in Peripheral Blood Neutrophils and Cytokine Levels in Healthy Volunteers and Patients With Pneumonia. *CHEST* 2001; 119: 1724-1729.

Textbook of Rheumatology; fifth ed. chapter 54. chapter written by Firestein Gary S. W.B. Saunders company 1997.

The Laboratory Rat. Chapter 17. Circulation. Chapter written by Livin V. D'Uscio, Juliane Kilo, Thomas F. Luscher. Edited by Georg J. Krinke. San Diego, Calif.; London: Academic, c2000.

Thomas WG. Regulation of angiotensin II type 1 (AT_1) receptor function. *Regulatory Peptides* 1999; 79:9-23

Thürnmann PA. Valsartan: a novel angiotensin Type 1 receptor antagonist. *Exp Opin Pharmacother* 2000; 1: 337-350.

Toth PP. Losartan reduces cardiovascular morbidity and mortality in patients with hypertension and left ventricular hypertrophy. *Curr Atheroscler Rep* 2003; 5: 339-340

Tracy KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF, Cerami AL. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* 1987; 330: 662-664.

Tsutamoto T, Wada A, Maeda K, Mabuchi N, Hayashi M, Tsutsui T, Ohnishi M, Sawaki M, Fujii M, Matsumoto T, Kinoshita M. Angiotensin II type 1 receptor antagonist decreases plasma levels of tumor necrosis factor alpha, interleukin-6 and soluble adhesion molecules in patients with chronic heart failure. *J Am Coll Cardiol* 2000; 35:714-721.

Turesson C, Jacobsson LTH. Epidemiology of extra-articular manifestations in rheumatoid arthritis (Review) *Scand J Rheumatol* 2004; 33: 65-72.

Uber A, Rettig R. Pathogenesis of primary hypertension—lessons from renal transplantation studies. *Kidney Int Suppl*. 1996; 55: S42-S45.

Ueda S, Meredith PA, Morton JJ, Connell JM, Elliott HL. ACE(I/D) genotype as a predictor of the magnitude and duration of the response to an ACE inhibitor drug (enalaprilat) in humans. *Circulation* 1998; 98: 2148-2153.

Ueki Y, Miyake S, Tominaga Y, Eguchi K. Increased nitric oxide levels in patients with rheumatoid arthritis. *J Rheumatol* 1996; 23: 230-236.

Ungerer M, Bohm M, Elce JS, Erdmann E, Lohse MJ. Altered expression of beta-adrenergic receptor kinase and beta 1-adrenergic receptors in the failing human heart. *Circulation* 1993 ; 87:454-463.

Ungerer M, Parruti G, Bohm M, Puzicha M, DeBlasi A, Erdmann E, Lohse MJ. Expression of beta-arrestins and beta-adrenergic receptor kinases in the failing human heart. *Circ Res* 1994; 74:206-213

Van Den Ouweland FA, Gribnau FWJ, Van Ginneken CAM, Tan Y, Van De Putte LBA. Naproxen kinetics and disease activity in rheumatoid arthritis: A within-patient study. *Clin Pharm Ther* 1988; 43: 79-85.

Van der Graaff WL, Prins AP, Niers TM, Dijkmans BA, van Lier RA. Quantitation of interferon gamma-interleukin-4-producing T cells in synovial fluid and peripheral blood of arthritis patients. *Rheumatology* 1999; 38: 214-220.

Van Snick J. Interleukin-6: an overview. *Annu Rev Immunol* 1990; 8: 253-278.

Vassalli P. The pathophysiology of tumor necrosis factors. *Annu Rev Immunol* 1992; 10: 411.

Verheggen PW, de Maat MP, Cats VM, Haverkate F, Zwinderman AH, Kluft C, Bruschke AV. Inflammatory status as a main determinant of outcome in patients with unstable angina, independent of coagulation activation and endothelial cell function. *Eur Heart J* 1999; 20: 567-574.

Verhoef CM, van Roon JA, Vianen ME , Bruijnzeel-Koomen CA, Lafeber FP, Bijlsma JW. Mutual antagonism of rheumatoid arthritis and hay fever; a role of type1/type2 T cell balance. *Annals of the Rheumatic Diseases* 1998; 57: 275-280.

Virdis A, Schiffrin EL.Vascular inflammation: a role in vascular disease in hypertension? *Curr Opin Nephrol Hypertens*. 2003;12:181-187.

Volanakis JE, Kaplan MH. Interaction of C-reactive protein complexes with the complement system. II. Consumption of guinea-pig complement by CRP complex: requirement for human C1q. *J Immunol* 1974; 113: 9-17.

Vroon A, Lombardi MS, Kavelaars A, Heijnen CJ. Changes in the G-protein-coupled receptor desensitization machinery during relapsing-progressive experimental allergic encephalomyelitis. *J Neuroimmunol.* 2003; 137: 79-86.

Vugrin D, Hood L, Taylor W, Laszol J. Phase II study of human lymphoblastoid interferon in patients with advanced renal carcinoma. *Cancer Treat Rep* 1985; 69: 817-820.

Waldmeier F, Flesch G, Muller P, Winker T, Kriemler HP, Buhlmayer P, De Gasparo M. Pharmacokinetics, disposition and biotransformation of [14C]-radiolabelled valsartan in healthy male volunteers after a single oral dose. *Xenobiotica* 1997; 27: 59-71.

Waldo AL, Camm AJ, deRuyter H, Friedman PL, MacNeil DJ, Pauls JF, Pitt CM, Schwartz PJ, Veltri EP. Effect of d-sotalol on mortality in patients with left ventricular dysfunction after recent and remote myocardial infarction. The SWORD Investigators. Survival With Oral d-Sotalol. *Lancet.* 1996; 348: 7-12. Erratum in : Lancet 1996; 348: 416.

Wallberg-Jonsson S, Cederfelt M, Rantapaa Dahlqvist S. Hemostatic factors and cardiovascular disease in active rheumatoid arthritis: an 8 year follow up study. ***J Rheumatol*** 2000*;* 27: 71-75.

Wang CH, Li SH, Weisel RD, Fedak PWM, Dumont AS, Szmitko P, Li RK, Mickle DAG, Verma S. C-reactive protein up-regulates angiotensin type 1 receptors in vascular smooth muscle. *Circulation* 2003; 107: 1783-1790.

Wang ZQ, Millatt LJ, Heiderstadt NT, Siragy HM, Johns RA, Carey RM. Differential regulation of renal angiotensin subtype AT_{1a} and AT_2 receptor protein in rats with angiotensin-dependent hypertension. *Hypertension* 1999; 33: 96-101.

Warren JS. Interleukins and tumor necrosis factor in inflammation. *Crit Rev Clin Lab Sci* 1990; 28: 37-59.

Wassmann S, Stumpf M, Strehlow K, Schmid A, Schieffer B, Böhm M, Nickenig G: Interleukin-6 induces oxidative stress and endothelial dysfunction by overexpression of the angiotensin II type 1 receptor. *Circ Res* 2004; 94: 534-541.

Watkins PB. The barrier function of CYP 3A4 and P-glycoprotein in the small bowel. *Adv Drug Delivery Red* 1997; 27: 161-170.

Weinblatt ME, Kremer JM, Bankhurst AD, Bulpitt KJ, Fleischmann RM, Fox RI, Jackson CG, Lange M, Burge DJ. A trial of etanercept, a recombinant tumor necrosis factor receptor: Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med* 1999; 340: 253-259.

Westhuyzen J, Healy H. Review: Biology and relevance of C-reactive protein in cardiovascular and renal disease. *Ann Clin Lab Sci* 2000; 30: 133-143.

Weyand CM, Goronzy JJ, Liuzzo G, Kopecky SL, Holmes DR Jr, Frye RL. T- cell immunity in acute coronary syndromes. *Mayo Clin Proc*. 2001; 76: 1011-1020.

Wink DA, Hanbauer I, Grisham MB, Laval F, Nims RW, Laval J, Cook J, Pacelli R, Liebmann J, Krishna M, Ford PC, Mitchell JB. Chemical biology of nitric oxide: regulation and protective and toxic mechanisms. *Curr Topics Cell Regulation* 1996; 34: 159-187.

Wolfe F, Mitchell DM, Sibley JT, Fries JF, Bloch DA, Williams CA, Spitz PW, Haga M, Kleinheksel SM, Cathey MA. The mortality of rheumatoid arthritis. *Arthritis Rheum* 1994; 37:481-494.

Wu L, Iwai M, Nakagami H, Li Z, Chen R, Suzuki J, Akishita M, de Gasparo M, Horiuchi M. Roles of angiotensin II type 2 receptor stimulation associated with selective angiotensin II type 1 receptor blockade with valsartan in the improvement of inflammation-induced vascular injury. *Circulation* 2001; 104: 2716-2721.

Xiao PR. β-adrenergic signaling in the heart : dual coupling of the β2-adrenergic receptor to Gs and Gi proteins. *Scie. STKE.* 2001; 104: 1-10.

Yamazaki M, Akiyama S, Niinuma K, Nishigaki R, Sygiyama Y. Biliary excretion of pravastatin in rats: contribution of the excretion pathways mediated by the canalicular multispecific organic anion transporter *Drug Met Disp* 1997; 25: 1123-1129.

Yamori Y, Ohtaka M, Nara Y. Vectorcardiograhic study on left ventricular hypertrophy in spontaneously hypertensive rats. *Japanese Circulation J* 1976; 40: 1315-1329.

Yasar U, Forslund-Bergengran C, Tybring G, Dorado P, Llerena A, Sjöqvist F, Eliasson E, Dahl M-L. Pharmacokinetics of losartan and its metabolite E-3174 in relation to the CYP 2C9 genotype. *Clin Pharmacol Ther* 2002; 71:89-98.

Yeh ETH. CRP as a mediator of disease. *Circulation* 2004; 109 [suppl II]: II-1 1-II-14

Yun C-H, Lee HS, Lee H, Rho JK, Jeong HG, Guengerich FP. Oxidation of the angiotensin II receptor antagonist losartan (DuP 753) in human liver microsomes. Role of cytochrome P4503A(4) in formation of the active metabolite EXP3174. *Drug Met Disp* 1995; 23: 285-289.

Zhang YX, Cliff WJ, Schoefl GI, Higgins G. Coronary C-reactive protein distribution: its relation to development of atherosclerosis. *Atherosclerosis* 1999: 145: 375-379.

Ziegler-Heitbrock HWL, Schlag R, Flieger D and Thiel D. Favorable response of early stage B CLL patients to treatmen with IFN-alpha 2. *Blood.* 1989; 73: 1426-1430.